

Washington State Department of Ecology

Environmental Assessment Program

Standard Operating Procedures for Resecting Finfish Whole Body, Body Parts or Tissue Samples

Version 1.1

Author – Patti Sandvik

Date -

Reviewer – Casey Deligeannis

Date -

QA Approval – William R. Kammin, Quality Assurance Officer

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Signatures on File

Please note that the Washington State Department of Ecology's Standard Operating Procedures (SOPs) are adapted from published methods, or developed by in-house technical and administrative experts. Their primary purpose is for internal Ecology use, although sampling and administrative SOPs may have a wider utility. Our SOPs do not supplant official published methods. Distribution of these SOPs does not constitute an endorsement of a particular procedure or method.

Any reference to specific equipment, manufacturer, or supplies is for descriptive purposes only and does not constitute an endorsement of a particular product or service by the author or by the Department of Ecology.

Although Ecology follows the SOP in most instances, there may be instances in which Ecology uses an alternative methodology, procedure, or process.

SOP Revision History

Revision Date	Rev number	Summary of changes	Sections	Reviser(s)
08/30/06	V1.0	SOP Publication		
10/19/10	V1.0	Three year review		
10/21/10	V1.0	QA approval, recertified		
03/21/14	V1.0	Three year review		
04/21/14	V1.1	QA approval, recertified		

Environmental Assessment Program

Standard Operating Procedure for Resecting Fish Tissue Samples.

1.0 Purpose and Scope

1.1 This document is the Environmental Assessment Program (EAP) Toxics Study Unit (TSU) Standard Operating Procedure (SOP) for resecting finfish tissue samples.

1.2 Washington Department of Ecology investigates the occurrence and concentrations of toxic contaminants in fish. This SOP is intended to provide consistent techniques that ensure the quality of tissue preparation (including whole finfish or other body parts) for the purpose of homogenizing samples for chemical analysis by an accredited analytical laboratory. Procedures for this SOP were adapted from the Environmental Protection Agency's (EPA) *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 Fish Sampling and Analysis Third Edition (2000)*.

2.0 Applicability

2.1 This procedure is to be followed by Ecology's TSU personnel when conducting finfish processing, and at the time of processing the fish in part or as a whole.

3.0 Definitions

3.1 Analyte – The substance or chemical constituent that is undergoing analysis. It is the substance being measured in an analytical procedure.

3.2 Processing Bench Sheet – A table, usually created in Excel®, used to plan and document sample processing data for each fish collected (Attachment 2).

3.3 Composite – Composite samples are homogeneous mixtures of equal amounts and types of tissue from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample.

3.4 Ecology – Washington State Department of Ecology.

3.5 DOH – Department of Health.

3.6 Dorsal fin – The main fin located on the back of fishes.

3.7 EAP – Environmental Assessment Program.

3.8 Lab Analysis & Tracking Plan – A table, usually created in Excel®, used to plan and document lab analyses of samples for single or multiple projects (Attachment 1).

- 3.9 LAR – Laboratory Analysis Required form.
- 3.10 MEL – Manchester Environmental Laboratories.
- 3.11 MSDS – Material Safety Data Sheets provide both workers and emergency personnel with the proper procedures for handling or working with a particular substance. MSDS include information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment and spill/leak procedures.
- 3.12 Operculum – Any one of the bony plates which support the gill covers of fishes.
- 3.13 OSWER – U.S. Environmental Protection Agency Office of Solid Waste and Emergency Response.
- 3.14 Otolith – One of many minute calcareous particles found in the inner ear of vertebrates. Fish species have three pairs of otoliths. The largest pair is used for aging.
- 3.15 PBDEs – Polybrominated diphenyl ethers
- 3.16 PCBs – Polychlorinated biphenyls.
- 3.17 PCDDs – Polychlorinated dibenzo-p-dioxins
- 3.18 PCDFs – Polychlorinated dibenzofurans
- 3.19 Pectoral fin – Either of the anterior pair of fins attached to the pectoral (mid) girdle of fishes.
- 3.20 QAPP – Quality Assurance Project Plan.
- 3.21 QC – Quality control.
- 3.22 Supplemental Bench Sheet for processing fillet and carcass tissue – A table, usually created in Excel®, for documentation of fish and parts of fish at different stages of processing. Data are used in mass balance equation to estimate contaminant concentrations in tissue that cannot be analyzed (Attachment 3).
- 3.23 Resecting – Surgical removal of all or part of an organ, tissue or structure.
- 3.24 WDFW – Washington Department of Fish and Wildlife.

4.0 Personnel Qualifications/Responsibilities

- 4.1 Because this procedure requires use of hazardous materials, training is required as per the Ecology *Chemical Hygiene Plan and Hazardous Materials Management Plan* (Section 1) (WA State Department of Ecology, 2011), which include Laboratory Safety Orientation, Job-Specific Orientation and Chemical Safety Procedures. Follow the Standard Operating Procedures in Section 16 of the *Chemical Hygiene Plan and Hazardous Materials Management Plan* for handling chemicals.

5.0 Equipment, Reagents, and Supplies

- 5.1 Deionized (DI) water and Nalgene wash squeeze bottles.
- 5.2 Forceps – Fine point, flat and rounded.
- 5.3 1 or 2 stainless steel fillet knives with 6-8 inch stainless steel blade.
- 5.4 Cleaver, hacksaw and blades.
- 5.5 Pliers (needle nose and regular) for pulling skin off. As well as wire cutters for cutting through bone and cartilage.
- 5.6 Surgical scalpels and box of new blades (stainless steel).
- 5.7 Fume hood.
- 5.8 Personal protection gear.
- 5.8.1 Talc-free nitrile exam gloves.
- 5.8.2 Gloves for solvents (see *Chemical Hygiene Plan and Hazardous Materials Management Plan* (Section 6) (WA State Department of Ecology, 2011).
- 5.8.3 Eye protection.
- 5.8.4 Full body apron.
- 5.8.5 Facial shield.
- 5.9 Sample jars – Short jars with Teflon lids (ICHEM certified 300 series, references for container cleaning procedures can be found in U.S. EPA OSWER directive 9240.0-05, *Specifications and Guidance for Obtaining Contaminant-Free Containers*, April 1990) (Figure 1). Determine the sizes of jars needed, based on the amount of tissue and analyses being performed, by using EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (EPA, 2000), Manchester Laboratory contacts and Manchester's Lab User's Manual (WA State Department of Ecology, 2008). The 4 oz (item#320-0125) and 2 oz (item#220-0060) jars are currently the

predominant choices. See full instructions for requesting sample containers from the Manchester's Lab User's Manual, and order based on the analytical plan prior to initial field sampling.

- 5.10 Create and print custom labels, or use labels provided with sample jars.
- 5.11 Heavy-duty aluminum foil (Reynolds Foodservice Foil 45.7cm x 152.4mm (624) and 38.1cm x 152.4mm (622)).
- 5.12 Sponges and scrub brushes.
- 5.13 Decontaminating fluids.
 - 5.13.1 10 % nitric acid. See Attachment 4 for MSDS and concentration grade before dilution. Dilute to 10% with DI water. Note – 5% nitric acid dilution should be used on Hobart grinders due to tin plated finish on grinder parts. If sampling for metals and plan to use Hobart grinder for tissue sample preparation, consider including equipment blank analysis to account for potential metals being introduced from tin plated grinder parts.
 - 5.13.2 Acetone (solvent) ACS, HPLC Grade, 99.9% min. See Attachment 5 for MSDS. See Section 4.1 of this document for safety requirements.
 - 5.13.3 Hexane (solvent) ACS HPLC Grade, 99.9% min. See Attachment 6 for MSDS. See Section 4.1 of this document for safety requirements.
 - 5.13.4 Liqui-Nox (biodegradable, phosphate-free, interfering-residue free, concentrated soap from Valconox, Inc.).
- 5.14 Small and large stainless mixing bowls (approximately 3 quarts and 13 quarts).
- 5.15 Medium and large stainless mixing spoons (approximately 8 inch and 15 inch).
- 5.16 Paper towels.
- 5.17 Garbage bags.
- 5.18 Zip seal bags – Pint, quart and gallon sizes.
- 5.19 Blue painter's masking tape.
- 5.20 Pens, pencils, pencil sharpener, permanent markers.
- 5.21 Kitchen Aid mixer.
- 5.22 5 Kitchen Aid grinding units (Ecology currently using model #K45SS) (Figure 2).

- 5.23 Hobart grinders (Ecology currently uses model #4812 and #4732A) (Figure 3).
- 5.24 Bench scale including spare 9-volt battery, standard weights and standards log book for pre and post accuracy checks (Figure 4).
- 5.25 Fish Measuring Board (Figure 5).
- 5.26 Magnifier (Ecology currently using an arm-mounted magnifier).
- 5.27 Polypropylene cutting board.
- 5.28 Sink and drainboard.



Figure 1. Illustration of 8 oz, 4 oz and 2 oz jars (WA State Department of Ecology, 2005).



Figure 2. Kitchen Aid with grinding unit.



Figure 3. Hobart grinding units (small unit #4812, large unit #4732A).



Figure 4. Scale Log Book, Standard Weights, spare 9v battery, and Bench Scale.

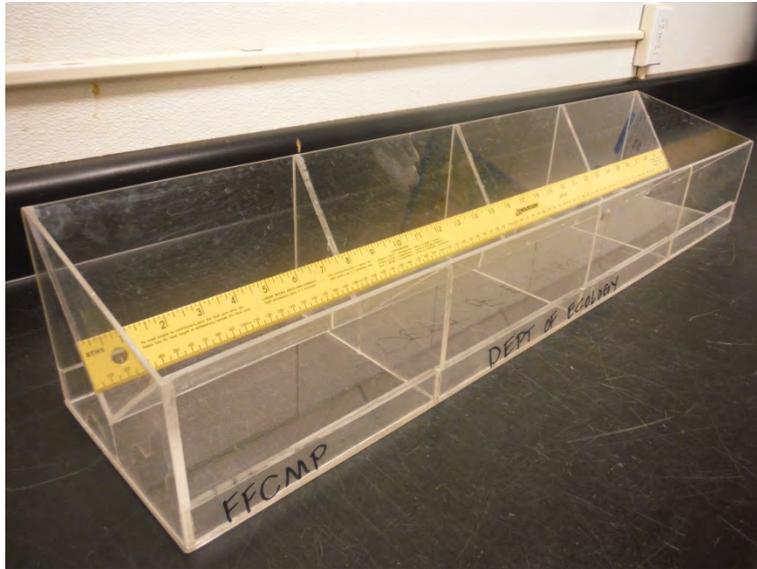


Figure 5. Fish Measuring Board.

6.0 Procedure

- 6.1 Decisions about how to process each fish are made by the project officer based on study objectives and the fish that were collected. Such decisions include whether to analyze individual fish or combine individuals to form composite samples, the number of fish used per composite sample, the size range of fish to be processed – either as individuals or as composite samples, whether the fish will be processed with or without the skin, and other related decisions. Such decisions are reflected in the Lab Analysis & Tracking Plan and Processing Bench Sheet.
- 6.2 Fish Processing Preparation.
 - 6.2.1 Formulate processing plan: timing, location, staff resources and equipment.
 - 6.2.2 Decide which jar sizes to order from Manchester Laboratory before processing is scheduled. See more comments for jar size and ordering under “Equipment, Reagents, and Supplies” section of this document. The general rule of thumb is to use the small 2 oz jar for mercury samples only, and the 4 oz jar for the remaining tissue analysis.

- 6.2.3 Determine how many jars are needed per process by consulting the Lab Analysis & Tracking Plan and counting one jar per type of analysis taken, plus one extra jar for archive samples. For example, if testing for pesticides (Pest), polychlorinated biphenyls (PCB), polybrominated diphenyl ether (PBDE), lipids and mercury (Hg), then two 4 oz jars and one 2 oz jar would be needed; a 4 oz jar each for “Pest PCB PBDE lipids” and “Archive,” and a 2 oz jar for “Hg.” Other combinations are possible. Therefore consult the Lab Analysis & Tracking Plan, count “one” for each analyte group, match this number to the number of jars needed and then add one more jar for the archived sample.
- 6.2.4 Prepare and print out a Lab Analysis & Tracking Plan spreadsheet for samples to be processed. See Section 7.1 and Attachment 1 of this document for instructions and an example of the Lab Analysis & Tracking Plan.
- 6.2.5 Prepare and print out a Processing Bench Sheet to record fish tissue processing data. See Section 7.2 and Attachment 2 of this document for instructions and an example of the Processing Bench Sheet.
- 6.2.6 Prepare and set up lab.
- 6.2.6.1 Make sure plenty of DI water is available and that the solvent/acid squeeze bottles are full. Check the main supply of acid and solvent solutions to make sure there are adequate amounts stocked for the processing season. There should also be a rinsate container (stainless steel bowl) and a funnel in the fume hood for solvent rinsing. Keep solvent and acids stored in proper lab cabinets under the counter until ready for use. See Ecology’s *Chemical Hygiene Plan and Hazardous Materials Management Plan* Section 16 Standard Operating Procedures (WA State Department of Ecology, 2011) for acid dilution and solvent handling procedures.
- 6.2.6.2 Put on protective equipment (apron, gloves, eye protection). Adhere to the safety procedures and training outlined in Section 4.1 of this document.
- 6.2.6.3 Decontaminate utensils for each individual fish and/or composite group before you begin, to avoid sample-to-sample contamination. Refer to Ecology’s *Chemical Hygiene Plan and Hazardous Materials Management Plan* Section 16 Standard Operating Procedures for decontamination procedures (WA State Department of Ecology, 2011). Decontaminated equipment needed for individual and composite samples include a fillet knife, scalpel, 3 quart SS bowl and large (approximately 8 inch) mixing spoon, and all the components of the Kitchen Aid grinding unit. Decontaminated equipment needed for individual and composite whole and carcass samples include a butcher knife, all the components of the Hobart grinding unit (5% nitric acid dilution should be used on Hobart grinders due to tin plated finish on grinder parts), a 13 quart SS bowl and an extra large (approximately 15 inch) mixing spoon. Include any equipment that will come in contact with the sample.
- 6.2.6.4 Clean lab space including dissecting areas, fume hood, equipment shelves, and floor (dust, sweep and wipe surfaces with damp cloth or sponge).

- 6.2.6.5 Pull out the fish samples to be processed per composite or group, to allow for thawing. Judge how long to thaw by the sizes of the specimens, by their field weight and length, or by visual inspection. Ideally, fish should be filleted while ice crystals are still present in the muscle tissue, just thawing them enough to insert a knife for filleting. Record the process date on the Lab Analysis & Tracking Plan spreadsheet and the Processing Bench Sheet. Include names or initials of processing crew at the top right of the Processing Bench Sheet.
- 6.2.6.6 Set up counter work area for ample space between resection processes. Five general stations are recommended: 1) desliming, descaling and rinsing in sink, 2) DNA, aging structures and sexing, 3) filleting or chopping, 4) grinding and mixing, and 5) record keeping, labeling and other documentation. Each station can then be utilized by multiple lab workers in an assembly line sequence. Care must be taken not to cross contaminate areas or samples, thus it is important to: frequently wash instruments, replace gloves, and reline each station with new foil dull-side-up after each process. The sink area, station 1 and 2, are considered the “dirty” areas, which are not acid and solvent cleaned as well as the equipment used in this area. Counter and equipment at station 3 and 4 are considered “clean” areas and therefore need to be covered, and instruments acid and solvent cleaned.
- 6.2.6.7 For station 1, clean and rinse thoroughly before starting on a new sample, to reduce the possibility of cross contamination.
- 6.2.6.8 For station 2, set up on foil the DNA sample vials, scale cards, aging structure envelopes and otolith tray – depending on WDFW aging structure requirements (See Ecology’s SOP #008 *Resecting DNA Samples and Aging for Finfish*). Possible equipment needed at this station includes a fillet knife, scalpel, scissors, forceps, pliers and container filled with DI water to rinse equipment. The equipment at this station doesn’t have to be decontaminated with acid and solvents. A bench light and magnifier are handy also for intricate maneuvers.
- 6.2.6.9 For station 3, set up on foil over chopping board the decontaminated fillet knife and scalpel for fillets and butcher knife for whole fish and carcasses. Tear off new foil large enough for each fish to be placed on for filleting procedure to be done after station 1 and 2’s resecting, scaling and DI rinsing.
- 6.2.6.10 For station 4, set up on foil the clean Kitchen Aid, decontaminated Kitchen Aid grinding unit, 3 quart bowl and large (approximately 8 inch) mixing spoon for fillets, or alternatively set up clean Hobart (decontaminated Hobart grinding unit including large pan that funnels tissue into grinder), 13 quart bowl and extra large (approximately 15 inch) mixing spoon for whole fish and carcasses. Note: Make sure when assembling the Hobart grinding unit that the grinding blade’s cutting edge (one-sided) sits flat against the holed-strainer plate. The Kitchen Aid blades are on both sides so either side can sit against the strainer plate. If compositing, tear off foil pieces large enough to wrap each ground sample mixture and then set these pieces aside near station 4. Also make sure

there is a label for each of these wrapped mixtures that identifies the sample, using the original fish ID.

- 6.2.6.11 For station 5, set out and review Lab Analysis & Tracking Plan and Processing Bench Sheet. Label DNA vials, scale cards, aging structure envelopes and otolith trays as described in Ecology’s SOP #008 *Resecting DNA Samples and Aging for Finfish*.
- 6.2.6.12 Pre-label fish tissue sample jars at station 5.
- 6.2.6.12.1 Label jars by using custom printed labels or labels provided with sample jars. If using labels provided with sample jars use a ballpoint pen or fine-tipped permanent marker to fill out needed info – allow time for ink to dry so it does not smear or run when wet. For composite samples, fill in the labels according to the “Sample Tag Labeling” illustration in Attachment 7 and stick on the side of the appropriate jar (Figure 5). If testing a composite for PBDE only, use “PBDE fish” labeling as illustrated in Attachment 7. For individual fish to be used in mercury trend analysis, label the 2 oz. jars similar except the “client/source” will be identified by the fish “Field ID #” taken from the Lab Analysis & Tracking Plan. An example could be for 10 fish collected from Potholes Reservoir and processed individually for “Hg Trend” analysis would be labeled “POT-1, POT-2, POT-3, ... etc.” (See Attachment 7). For all jars, write the abbreviated Field ID (listed in the Lab Analysis & Tracking Plan) and last two digits of sample number on the jar lids (Figure 6), using a fine tipped permanent marker.

I - CHEM	90g
CLIENT/SOURCE FFCMP14	<input type="checkbox"/> GRAB <input checked="" type="checkbox"/> COMPOSITE
SITE NAME LOONLMB	DATE/TIME 10/26/14
SAMPLE # 1401015-xx	PRESERVATIVE
ANALYSIS PEST-PCB-PBDE-LIPIDS	COLL. BY

Figure 5. Freshwater Fish Contaminant Monitoring Program example label for composite fish tissue jar.

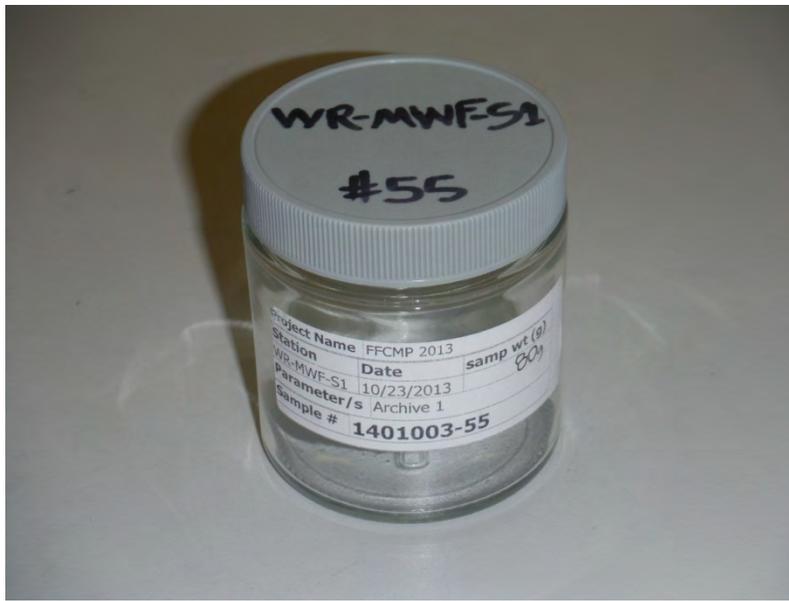


Figure 6. Example of sample jar lid information and custom printed label.

6.3 Fish Processing Resection.

6.3.1 The resection process steps described below are intended to be complete within each category of fillet, whole and carcass. Turn to the appropriate subheading of either “Individual fish resection” or “Preparation of composite sample fish resection” and read the brief introduction. Then follow the procedures outlined for fillet, whole or carcass, depending on the project’s objectives for each fish sample or group of samples collected.

6.4 Individual fish resection.

6.4.1 It is essential that the weights of individual homogenates be sufficient to perform all necessary analyses. Generally, lab analyses for groups of organic compounds (e.g. PCBs, lipids, chlorinated pesticides) require a minimum of 30 grams of homogenized tissue. Lab analyses for mercury and other metals generally require a minimum of 5 grams. Check with project officer and/or laboratory for minimum amounts needed for each analysis or group of analyses.

6.4.2 Although there is a 30-gram minimum weight for organics and a 5-gram minimum weight for mercury, the weights may need to be doubled or more. The extra weight is intended to provide sufficient sample material to analyze for all recommended target analytes at required detection limits, meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike and matrix spike duplicate samples, and allow for reanalysis if the QC control limits are not met or if the sample is lost.

6.4.3 Therefore, check the Lab Analysis & Tracking Plan to determine which analytes are being tested and the number of jars needed. Thus a determination can be made whether to use one fillet side or both fillet sides of the individual fish in order to have enough tissue for homogenizing.

- 6.5 Fillet.
- 6.5.1 Unwrap an individual fish, keeping it on foil at station 2, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Look to see that the sample was properly preserved during shipment, tissue wasn't destroyed, and correct species were identified, and note any gross morphological abnormalities. Double check to make sure that the fish field identification matches Processing Bench Sheet and Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Bench Sheet. If the field weight and/or length are not recorded, weigh and measure the fish during this inspection time, writing the measurements on the Processing Bench Sheet (See Ecology's SOP #009 *Field Collection, Processing and Preservation of Finfish Sample*).
- 6.5.2 If needed, remove DNA samples prior to continuing next steps to ensure intact genetic sample is collected. Aging structures such as otoliths, operculum, and spines should be removed after the fillet sample is taken, to prevent contamination of sample tissue when removing fillets (See Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*).
- 6.5.3 At station 1, remove slime and scales from fish that have scales by scraping from the tail to the head using the blade edge or the back side of a large knife, and rinsing periodically under cold running water. Be careful not to thaw the fish too much. Small specimens thaw very quickly, especially in water. After the fish is scaled, rinse it with DI water and, at station 3, place on a new piece of foil (dull side up) with the accompanying field ID tag. Rinse the scaling equipment with DI water between fish if other individual fish are to be resected.
- 6.5.4 At station 1, remove slime from fish without scales by scrubbing then rinsing with DI water, and place fish on new piece of foil (dull side up) with accompanying field ID tag over station 3. To remove the skin, first outline the area where skin is to be removed by making an incision just through the skin. Then loosen skin just behind the gills and pull it off with pliers, pulling from the head towards the tail. Rinse the skinning equipment with DI water between fish samples, if other fish are to be resected.
- 6.5.5 Fillet fish at station 3 while ice crystals are still present in the muscle tissue, using the illustrated procedure in Attachment 8 and/or under the direction of an experienced person. Any dark muscle tissue in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Include the belly flap portion if possible. Avoid bones while filleting.
- 6.5.5.1 Care must be taken to avoid contaminating fillet tissues with material and fluid released from inadvertent slicing into the internal organ cavity. If the fillet tissue is contaminated by materials released from the internal cavity and/or organs during resection, the tissue may be eliminated as a sample or, alternatively, the fillet tissue should be rinsed in contaminant-free, DI water. Make a note on the Processing Bench Sheet if this occurs.

- 6.5.5.2 Fillets should be cut into smaller pieces to facilitate homogenization in the Kitchen Aid grinder. Smaller pieces may be cut as the fillet is removed from the fish. Prior to grinding tissue, set up a lab scale with a small piece of foil, dull side up. Tare the scale so it reads zero. Place the cut up pieces of fillet tissue on the foil. Weight the fillet to the nearest gram and record the weight. Also record which side the fillet was taken from (i.e. right, left, or both) and whether the fish was processed with skin on or off.
- 6.5.5.3 Totally wrap the tissue within the foil and secure a sample identification label to it. Place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization is done. Return the carcass to station 2, ("dirty" area), for age structure removal and for determining the sex.
- 6.5.6 Homogenize the fish tissue at station 4 using the previously decontaminated Kitchen Aid grinder, stainless steel bowl and spoon. Grind and then mix the tissue until it appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together by hand, and then mix the two halves together. **Repeat the grinding and mixing two more times.** No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.
- 6.5.7 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, and increases the risk of losing the sample due to jar breakage. 90 grams maximum is enough for the 4 oz. jars and roughly 50 grams or $\frac{3}{4}$ full maximum is enough for the 2 oz. jars. Record the homogenate weight in the corner of the jar label and on the lab tracking sheet. If only using the individual fish tissue for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return it to the freezer to be used with other processes such as compositing.
- 6.5.8 At station 2, remove aging structures (otoliths, operculum and spines) appropriate for the species. Record appropriate information on the Processing Bench Sheet per Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*.
- 6.5.9 Determine the sex of fish by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, a second incision should be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the species can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture.

Record the sex of each fish on the Processing Bench Sheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F for unsure.

- 6.5.10 Verify and document that the Lab Analysis & Tracking Plan, Processing Bench Sheet, and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.
- 6.5.11 Store jars in freezer (≤ -20 °C) with lids secure for transport to lab. See MEL user's manual for instructions for shipping samples to the lab (WA State Department of Ecology, 2008).
- 6.6 Whole fish.
 - 6.6.1 Unwrap an individual fish, keeping it on foil at station 2, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Look to see that the sample was properly preserved during shipment, tissue wasn't destroyed, and correct species identified, and note any gross morphological abnormalities. Double check to make sure that the fish field identification matches the Processing Bench Sheet and the Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Bench Sheet. If the field weight and/or length are not recorded, weigh and measure the fish during this inspection time, writing the measurements on the Processing Bench Sheet (See Ecology's SOP #009 *Field Collection, Processing and Preservation of Finfish Samples* for weighing and measuring techniques).
 - 6.6.2 If needed, remove DNA sample and aging structures appropriate for the species (See Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*).
 - 6.6.3 Slime, scales and/or skin might be included in whole fish analysis. Therefore, processors must check with the project officer and/or the lab plan and Processing Bench Sheet. If removing scales, remove slime from fish that have scales at station 1 by scraping from the tail to the head using the blade edge or the back side of a large knife. Be careful not to thaw the fish too much. Small specimens thaw very quickly, especially in water. After the fish is scaled, rinse the fish with DI water. At station 3, place the fish on a new piece of foil (dull side up) with the accompanying field ID tag. Rinse the scaling equipment with DI water between fish, if other individual fish are to be resected. To remove the skin, loosen it just behind the gills and pull it off with pliers. Rinse the skinning equipment with DI water between fish samples if other individual fish are to be resected.
 - 6.6.4 For fish where scales or skin are not removed, remove slime at station 1 by scrubbing then rinsing with DI water. Then place the fish on new piece of foil (dull side up), with accompanying field ID tag at station 3.

- 6.6.5 At station 2, remove structures for aging (otoliths, operculum and/or spines) appropriate for the species. Record appropriate information on the Processing Bench Sheet per Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*.
- 6.6.6 If needed, determine the sex of fish at station 2 or station 3 by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, a second incision should be made on the other side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the species can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture. Record the sex of each fish on the Processing Bench Sheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F for unsure.
- 6.6.7 If using the large Hobart grinder (model #4732A) to homogenize whole fish you can skip to section 6.6.9 in this SOP (first, ensure intact whole fish will fit down Hobart grinder chute). Otherwise, fish should be chopped into pieces small enough to facilitate homogenization in the Kitchen Aid and Hobart grinders. Chop, cut or saw fish using appropriate tools (cleaver, knives, hacksaw) at station 3 while ice crystals are still present in the muscle tissue. Safety glasses are recommended. Place the fish pieces on aluminum foil that is dull side up.
- 6.6.8 Totally wrap the tissue within the foil and secure a sample identification label to it, place the wrapped tissue in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants, and frozen until homogenization is done. Write on the Processing Bench Sheet whether the fish is processed with the skin on or off.
- 6.6.9 Homogenize fish tissue at station 4 using a previously decontaminated grinder, stainless steel bowl and spoon. (Whole fish are typically ground using the Hobart grinder). Grind and then mix until the tissue appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together by hand and then mix the two halves together. **Repeat the grinding and mixing two more times.** No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.
- 6.6.10 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. 90 grams maximum is enough for the 4 oz. jars, and 50 grams or $\frac{3}{4}$ full maximum is enough for the 2 oz. jars. Record the homogenate weight in corner of the jar label and on the Lab Analysis and Tracking Plan. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return it to the freezer to be used with other processes such as compositing.

- 6.6.11 Verify and document that the Lab Analysis & Tracking Plan, Processing Bench Sheet, and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.
- 6.6.12 Store jars in freezer (≤ -20 °C) with lids secure for staging transport to lab. See MEL user's manual for instructions for shipping samples to the lab (WA State Department of Ecology, 2008).
- 6.7 Carcasses.
- 6.7.1 The purpose of processing fish carcasses and fillets separately is to determine the ratio of contaminant concentration in fillets to whole fish, in order to estimate concentrations in fillet tissue when only whole-fish data are available. Determining this ratio requires a mass balance approach using concentration and weight data from fillets and the remaining carcass. To account for changes in weight of each fish during processing, (e.g. weight loss from scale removal), the weight of each fish and its various tissues should be recorded at selected steps in the processing procedure.
- 6.7.2 The fillets and carcasses from the same fish sample are treated as two separate samples. Therefore, cleaned and decontaminated equipment is needed for each sample. Refer to Ecology's *Chemical Hygiene Plan and Hazardous Materials Management Plan* Section 16 Standard Operating Procedures for decontamination procedures (WA State Department of Ecology, 2011).
- 6.7.3 Prepare the Supplemental Bench Sheet for processing fillet and carcass tissue, in addition to the Lab Analysis & Tracking Plan and regular Processing Bench Sheet. Fill in the site location name, fish species, fish field ID and weight for matching individual fish (See Section 7.3 and Attachment 3 in this document for the Supplemental Bench Sheet for processing fillet and carcass tissue instructions and example).
- 6.7.4 At station 2, unwrap an individual fish, keeping it on foil, and perform a simple inspection to make sure the specimen will not compromise the representativeness and accuracy of the data in any way. Look to see that the sample was properly preserved during shipment, that tissue wasn't destroyed, that correct species were identified, and note any gross morphological abnormalities. Double check to make sure that the fish field identification matches the Processing Bench Sheet and the Lab Analysis & Tracking Plan identification. Be sure to document any findings on the Processing Bench Sheet. If the field length is not recorded, measure the fish during this inspection time, documenting the measurements on the Processing Bench Sheet (See Ecology's SOP #009 *Field Collection, Processing and Preservation of Finfish Samples* for measuring techniques).
- 6.7.5 Weigh whole fish on new foil, dull side up, on a tared bench scale. Record weight to the nearest gram on the Supplemental Bench Sheet for processing fillet and carcass tissue.

- 6.7.6 Remove DNA sample and aging structures appropriate for the species, except the otoliths, which will be removed after the fillet sample is taken (See Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*).
- 6.7.7 At station 1, scale and remove slime from fish that have scales, by scraping from the tail to the head using the blade edge or the back side of a large knife and rinsing periodically under cold running water. Be careful not to thaw the fish too much. Small specimens thaw very quickly especially in water. After the fish is scaled, rinse the fish with DI water and place on a new piece of foil (dull side up) with the accompanying field ID tag over at station 3. Rinse the scaling equipment with DI water between fish if other individual fish are to be resected.
- 6.7.8 Weigh whole fish again after scales and DNA sample are removed. Record the weight to the nearest gram on the Supplemental Bench Sheet for processing fillet and carcass tissue. Use new foil dull side up, or the same foil used before scale and DNA resection.
- 6.7.9 Fillet fish at station 3 while ice crystals are still present in the muscle tissue, using the illustrated procedure in Attachment 8 and/or under the direction of an experienced person. Any dark muscle tissue in the vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass. Include the belly flap portion if possible. Avoid bones while filleting.
- 6.7.10 Care must be taken to avoid contaminating fillet tissues with material released from inadvertent slicing into the internal organ cavity. If the fillet tissue is contaminated by materials released from the internal cavity and/or organs during resection, the tissue may be eliminated as a sample or, alternatively, the fillet tissue should be rinsed in contaminant-free, DI water and blotted dry. Notation should be made in the Processing Bench Sheet.
- 6.7.11 Fillets should be cut into smaller pieces to facilitate homogenization of the Kitchen Aid grinder. Prior to grinding tissue, set up the lab scale with a small piece of foil dull side up on the scale. Tare the scale so it reads zero. Place the cut up pieces of fillet tissue on the foil. Weigh the fillet to the nearest gram and record the weight of both fillets on the Supplemental Bench Sheet for processing fillet and carcass tissue.
- 6.7.12 Totally wrap the fillets within the foil and secure a sample identification label to it, and place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization.
- 6.7.13 Remove otolith and aging structures (i.e. operculum/spines) appropriate for the species at station 2 (See Ecology's SOP #008 *Resecting DNA Samples and Aging for Finfish*). Record otolith tray number, cell number and aging structure type and ID number in Processing Bench Sheet.

- 6.7.14 Weigh otoliths and other aging structures (i.e. operculum/spines). Record any measurable weight on the Supplemental Bench Sheet for processing fillet and carcass tissue.
- 6.7.15 Weigh remaining carcass on new foil dull side up. Record weight to the nearest gram in the Supplemental Bench Sheet before processing the fillet and carcass tissue.
- 6.7.16 Determine sex of fish by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pectoral fins. If necessary, a second incision should be made on the other side of the fish from the initial point of the first incision toward the dorsal fin. The resulting flap should be folded back to observe the gonads. Ovaries have a granular texture and depending on the specie can range from orange/red to dark green/blue or even whitish in color. Testes appear creamy off-white and have a smooth texture. Record the sex of each fish on the Processing Bench Sheet using M for male, F for female, U for unknown. Add a question mark (?) after M or F for unsure.
- 6.7.17 If using the large Hobart grinder (model #4732A) to homogenize carcass you can skip to section 6.7.19 in this SOP (first, ensure fish carcass will fit down Hobart grinder chute). Otherwise, chop carcass at station 3, using a butcher knife, while ice crystals are still present in the muscle tissue. Safety glasses are recommended. Fish pieces should be chopped small enough to facilitate homogenization of the Hobart grinder. Place the fish pieces on a piece of aluminum foil that is dull side up.
- 6.7.18 Totally wrap the tissue within the foil and secure a sample identification label to it, place the packet in the freezer (≤ -20 °C) for temporary (less than one day) storage to keep the tissue free from contaminants and frozen until homogenization.
- 6.7.19 Homogenize fish carcass pieces at station 4 using a previously decontaminated Hobart grinder, stainless steel bowl and spoon. Grind and then mix until the tissue appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together by hand and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.
- 6.7.20 Fill sample jars with adequate amount of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. 90 grams maximum is enough for the 4 oz. jars and 50 grams or $\frac{3}{4}$ full maximum is enough for the 2 oz. jars. Record the homogenate weight in the corner of the jar label and on the Lab Analysis and Tracking Plan. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil and return to the freezer to be used with other processes such as compositing.

- 6.7.21 Verify and document that the Lab Analysis & Tracking Plan, Processing Bench Sheet, Supplemental Bench Sheet and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.
- 6.7.22 Store jars in freezer (≤ -20 °C) with lids secure for staging transport to lab. See MEL User's Manual for instructions for shipping samples to the lab (WA State Department of Ecology, 2008).
- 6.7.23 Homogenize fish fillets at station 4 using the previously decontaminated Kitchen Aid grinder, stainless steel bowl and spoon. Grind and then mix until the tissue appears to be of a consistent color and texture. If there is a large amount of tissue, divide the ground tissue into quarters, mix the opposite quarters together by hand and then mix the two halves together. Repeat the grinding and mixing two more times. No chunks of tissue or skin should remain in the sample homogenate. Poorly homogenized tissue may not be extracted or digested efficiently and could bias the analytical results. If necessary, repeat the grinding and mixing again. Each sample should be ground and mixed three times at minimum.
- 6.7.24 Fill sample jars with adequate amounts of homogenate. Do not pack the jars too full because the homogenate expands when it freezes, which increases the risk of losing the sample due to jar breakage. 90 grams maximum is enough for the 4 oz. jars and 50 grams or $\frac{3}{4}$ full maximum is enough for the 2 oz. jars. Record homogenate weight in the corner of jar label and on the lab tracking sheet. If only using the individual fish for one process, then move on to the next step. Otherwise, weigh the rest of the ground tissue, note this on the sample identification label, rewrap the tissue in foil, and return to the freezer to be used with other processes.
- 6.7.25 Verify and document that the Lab Analysis & Tracking Plan, Processing Bench Sheet, Supplemental Bench Sheet and all labeling is complete and accurate. Be sure to write down the process date and crew name initials.
- 6.7.26 Store jars in freezer (≤ -20 °C) with lids secure for transport to lab. See MEL user's manual for instructions for shipping samples to the lab (WA State Department of Ecology, 2008).
- 6.8 Preparation of composite sample.
- 6.8.1 Composite samples are prepared using equal-weight aliquots of processed tissue from one or more individual fish. When using equal-weight aliquots from each fish, the amount of tissue available from the smallest fish determines the size, or weight, of the aliquot to be used from each fish making up the composite sample. When using small fish to create a composite sample, first determine if there is adequate tissue combined from all fish designated for the composite to meet analytical needs. Measure or estimate the amount of tissue available from the smallest fish then multiply by the number of fish in composite to find the amount of tissue available for the required analyses. If the

combined amount available is greater than or equal to the amount of tissue needed for analyses (including sufficient sample material to analyze for all recommended target analytes at required detection limits; meet minimum QC requirements for the analyses of laboratory duplicate, matrix spike and matrix spike duplicate samples; and allow for reanalysis if the QC control limits are not met or if the sample is lost), then proceed with processing individual samples and creating the composite sample as described below. If there is not enough tissue, then determine whether to increase the number of fish used in the composite sample or limit the number of lab analyses.

- 6.8.2 Organic testing requires a minimum of 30 grams of homogenized tissue and mercury testing requires a minimum of 5 grams. Check the Lab Analysis & Tracking Plan to determine which analytes are being tested and the number of jars needed.
- 6.9 Fillet, whole and/or carcass tissue compositing.
 - 6.9.1 Follow procedures for individual fish tissue resection above (fillet, whole fish, carcasses), but grind and mix tissue from each fish only twice before wrapping in foil, marking the weight of the tissue on it and storing it in the freezer. (The third homogenization and mixing will be done as the homogenates from individual fish are combined, ground, and mixed together).
 - 6.9.2 Combine equal weights of tissue from individual homogenates (fillet, whole or carcass). If individual homogenates have been frozen, they should be thawed partially and rehomogenized prior to compositing. Any associated liquid should be maintained as a part of the sample. Record the aliquot weight to the nearest gram on the Lab Analysis & Tracking Plan.
 - 6.9.3 Mix all individual homogenate aliquot portions together until completely homogenized. **Grind and mix one more time.** The tissue should be ground three times and mixed four times at minimum by the time tissue from the individual fish are composited and ready to put in containers to be sent to the lab.
 - 6.9.4 Continue following the instructions for processing individual fillet, whole fish or carcasses.

7.0 Records Management

- 7.1 Lab Analysis & Tracking Plan – This spreadsheet tracks information about tissue samples from field collection through final lab analysis. This tool is used to organize, plan, coordinate, and track sample characteristics for single or multiple projects. The spreadsheet is used to: determine which lab analyses are done on which samples, determine costs for analyses and QA/QC requirements, document lab and field identification codes, and document other sample and plan characteristics. The structure of the spreadsheet may vary depending on the type of project(s) and the objectives, and can be tailored to suit the user. Table 1 lists the fields used in this form. Some fields are

required for all projects (R), while others are suggested (S) or optional (O). See Attachment 1 for an example.

- 7.1.1 The Lab Analysis & Tracking Plan form is prepared at the beginning of field collections for each project or season. As fish are collected during the project, information is entered into the form for the project, site, species, collection date, and number of fish available for each species. As sampling and planning progresses, the fields indicating the desired lab analyses can be populated. Doing so produces numbers and costs of analyses, allowing project staff to track anticipated costs and make changes as needed.
- 7.1.2 A hardcopy of the spreadsheet is used to record data while processing fish. As samples are processed, sample information is recorded on the spreadsheet (e.g. the sample process date, aliquot used per fish, etc.). These handwritten data are transferred to the electronic version at regular intervals so project staff can determine the status of fish collections and sample processing. When entering documentation from the hard copy into the master electronic lab tracking plan, write the word *Entered, the date, and your initials* on each hard copy of the lab tracking plan.
- 7.1.3 A template of the Lab Analysis & Tracking Plan is located on Ecology Intranet at Y:\SHARED Files\TSU Fish\2013 Fish\filename of template (see Attachment 1). The name of the file should include the project sampling year, the words or abbreviation indicating “Lab Tracking Plan”, and the version number, (e.g. 2013 Lab Analysis & Tracking Plan.xlsx).

Need	Field Title	Field Description
R	Project 1 pre-assigned work order #	Pre-assigned 7 digit MEL work order (example: FFCMP work order #1401003-xx).
R	Project 2 pre-assigned work order #	Pre-assigned 7 digit MEL work order (example: Hg Trends work order #1401009-xx).
R	Site	Name of the waterbody fish was collected from.
R	Specie	Abbreviated species of fish. Example: LMB (largemouth bass), MWF (mountain whitefish).
R	Collect Date	Date fish was collected, m/d/yy.
R	# Fish in Comp	Number of fish in composite.
R	LAR Field Station ID	Unique identification given to each sample. Name used on LAR (Laboratory Analysis Required) form.
R	MEL Lab #	Unique 2 digit sample ID added on end of work order # during fish processing (typically #s 01-99, example #1401003-01).
R	Process Date	Date the fish was processed, m/d/yy.
R	Aliquot per Fish (g)	Weight in grams per fish in equal aliquots for composite.
R	Skin: off or on	Skin is either removed or left on when processing.
R	Comment	Additional observations, instructions and procedure documentation.
S	Project 1	Example: FFCMP (Freshwater Fish Contaminant Monitoring Program).
S	Project 2	Example: Hg Trends.
S	Analyte Group 1	Example: Pest PCB PBDE lipid.
S	Analyte Group 2	Example: PCB congener.
S	Analyte Group 3	Example: Hg (mercury).
S	Analyte Group 4	Example: PCDD/F
S	MEL Lab Dup	MEL lab duplicates indicated for project.
S	Contract Lab Dups	Contract lab duplicates indicated for project.
S	MS/MSD	MEL lab matrix spikes and standards indicated for project.
S	# Fish Avail	Total number of fish collected.
O	Sort	A column allowed for entries from which to sort specifications as needed.
O	Analytical Group 1 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: Pest PCB PBDE lipid (g).
O	Analytical Group 2 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: PCB congener (g).
O	Analytical Group 3 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: Hg (g).
O	Analytical Group 4 sample weight	Weight in grams of sample sent to MEL for these analytes. Example: PCDD/F (g).
O	Archive	Weight in grams of sample archived. Ocassionally a second archive jar is retained for future needs.
O	Samples to Lab date	Date processed fish samples were sent to MEL.
O	Fillet+Carcass	Special process indicated when marked with an F or C, (Fillet/ Carcass).
O	Process Batch (H-Hg trends; F-FFCMP)	Example of study/user specific documentation. This was created for prioritizing analysis by study type.

Table 1. Lab Analysis and Tracking Plan field titles and descriptions. Field entries are designated as required (R), suggested (S) or optional (O). Additional fields may be used as needed per study or user preference.

- 7.2 Processing Bench Sheet – This spreadsheet is used to document field and processing data for individual fish. Such information includes individual fish identification coding, composite sample designation, fillet weights, sex, age structure cross references, and general comments about the fish or its processing. The structure of the spreadsheet may vary depending on project(s) and objectives and can be tailored to suit the user. Table 2 lists fields used in this form. Some fields are required for all projects (R), while others are suggested (S) or optional (O). See Attachment 2 for an example.
- 7.2.1 A hardcopy of the spreadsheet is used to record data while processing fish. As samples are processed, sample information is recorded on the spreadsheet. These handwritten data are transferred to the electronic version at regular intervals, so project staff can determine the status of fish collections and sample processing. When entering information from the hard copy into the master electronic lab tracking plan, write the word *Entered, the date, and your initials* on each hard copy of the Processing Bench Sheet.
- 7.2.2 A template of the Processing Bench Sheet is located on Ecology Intranet at Y:\SHARED Files\TSU Fish\2013 fish\filename of template (see Attachment 2). The name of the file should include the project sampling year, the words or abbreviation indicating “Bench Sheet” and the version number, (e.g. 2013 Fish Processing Bench Sheet 2.xls).

Need	Field Title	Field Description
R	Site	Name of the waterbody fish was collected from.
R	ECY Field ID	Identification (number or combination given to fish when collected in the field).
R	Species Code	Abbreviated species of fish. Example: LMB (largemouth bass), MWF (mountain whitefish).
R ¹	WDFW DNA ID	Identification of the vial that the DNA sample clip is stored, (i.e. CD05-##).
R	Total Length (mm)	Total length of fish in millimeters.
R	Weight (g)	Weight of fish in grams.
R	Collect Date	Date fish was collected.
R	Process Date	Date the fish was processed.
R	Fillet Weight (g)	Fillet weight of fish in grams if filleted.
R	L, R, or B fillet	L, R, or B for left, right, or both side(s) of fish that was filleted.
R	Skin Status	On or off for skin when processing.
R	Sex	Sex of fish - Male = M, Female = F, Unidentified = U.
R ²	Scale Card #	Number of scale card.
R ²	Scale #	Individual fish identification, which can be the same as the Field ID.
R ²	Otolith Tray #	Number of otolith tray.
R ²	Otolith Cell #	Otolith cell number.
R ²	Opercle or Spines taken Y/N	Indicates if operculum or spines were collected.
R	Comment	Additional observations, instructions and procedure documentation.
S	WDFW Field ID	WDFW field ID if collected by WDFW (if available).
S	DNA Taken?	Yes or No (Y or N) confirmation that the DNA tissue sample was collected.
S	Collect Method	Abbreviated collection method. See Collection Methods Abbreviations ^a .
S	Fish Age	Blank until WDFW results return indicating age of fish.
S	Fin Clipped?	For ID as hatchery origin. Usually salmonids, rainbow trout, cutthroat trout.
S	LAR Field Station ID	Unique identification given to each sample. Name used on LAR (Lab Analysis Required) form.
S	MEL Lab ID #	Unique 2 digit sample ID added to end of work order # during fish processing (typically #s 01-99. example: 1401003-25).
O	Composit Group	Group of fish that were processed together to create a composite sample.
O	Fork Length	Length of fish from nose to crescent in tail.

Table 2. Processing Bench Sheet field titles and descriptions.

The appended Table a. Collection Method Abbreviations describes fish collection method abbreviations for the Collect Method field of Processing Bench Sheet. Field entries are designated as required (R), required only if DNA sample is collected (R¹), required only if fish is aged (R²), suggested (S) or optional (O). Additional fields may be used as needed per study or user preference.

Code	Agency or Collection Method
ecy	Ecology
dfw	WA Dept of Fish and Wildlife
epa	U.S. Environmental Protection Agency
Tri	Tribe
UW	University of Washington
E	Boat electroshock
G	Gillnet
A	Angling
T	Trolling
BP	Backpack electrofishing
BS	Beach seine
NPMP	Northern Pike minnow Program

Table a. Collection Methods Abbreviations. Combine the agency code with the collection method code to create “collection method code” (e.g. “ecy-E”).

7.3 Supplemental Bench Sheet – is for documenting the weights of the different tissue parts while processing fillet and carcass tissue, in order to determine the ratio of contaminant concentration in fillets to whole fish for estimating concentrations of contaminants in fillet tissue when only whole-fish data are available.

7.3.1 The Supplemental Bench Sheet is located on Ecology Intranet at Y:\SHARED Files\TSU Fish \2013 fish\filename of template (see Attachment 3). A copy may also be created from Attachment 3 of this document. Enter all hard copy documentation into the master electronic Supplemental Bench Sheet for processing fillet and carcass tissue. Write the word *Entered, the date and your initials* on each hard copy of the Supplemental Bench Sheet that you entered all the data from.

8.0 Quality Control and Quality Assurance Section

8.1 Verify that all information is filled out on the bench sheet. There should not be any blank cells under any fields except fish age, since that is determined by WDFW and filled in later.

8.2 Verify that the values for weight of homogenate sample, date of processing and Field ID are complete in the Lab Analysis & Tracking Plan.

8.3 Verify that all the information is filled out on the Supplemental Bench Sheet for processing fillet and carcass tissue. There should not be any blank cells except possibly the field labeled “other.”

8.4 Verify all hard copy documentation on the Processing Bench Sheet and Supplemental Bench Sheet for processing fillet and carcass tissue accuracy. Cross check hard copies against electronic versions to verify data.

9.0 Safety

- 9.1 Fish processing should be conducted only by or under the supervision of someone with experience.
- 9.2 Gloves are required for fish processing to avoid exposure to pathogens and chemicals, and to avoid sample contamination. Hands should be cleaned using soap and clean water after completing work or any time hands become soiled during the process. Gloves should be changed whenever they get torn, punctured, or anytime used gloves are removed from hands.
- 9.3 The use of nitric acid, acetone and hexane requires training as per the *Chemical Hygiene Plan and Hazardous Materials Management Plan* (Section 1) (WA State Department of Ecology, 2011), which includes a Laboratory Safety Orientation, Job-Specific Orientation and must know Chemical Safety Procedures and follow the “Basic Lab Rules” (Section 2). Glove, safety glasses and a full body protective apron is required. MSDS can be found in this document and in a MSDS manual in the lab room where the process takes place.
- 9.4 Extreme care must be taken when using all knives, not only for your safety, but also for others that may be working in close proximity. Verify that the first aid kit is available in the lab room and the contents are complete. Contact the room supervisor and/or the safety officer if any accident occurs, if first aid supplies are inadequate, if chemical spills occur, or for any other need or questions. The names and numbers of the room supervisor are posted in the room. In extreme emergency call 911. Work with a “buddy” if possible or notify a co-worker of your lab work plans, and put them on your calendar.

10.0 References

- 10.1 Wikipedia. 2014. The free encyclopedia. <http://en.wikipedia.org/wiki/DNA>. Accessed April 2014.
- 10.2 Fisher Science. 2006. Supplier of scientific equipment, supplies, chemicals and furniture. <https://www1.fishersci.com/index>. Accessed April 2014.
- 10.3 U.S. EPA (Environmental Protection Agency). 2000. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 Fish Sampling and Analysis. 3rd ed. <http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/index.cfm>. Accessed April 2014.
- 10.4 VWR. 2006. Supplier of scientific equipment, supplies, chemicals and furniture. <https://us.vwr.com>. Accessed April 2014.

- 10.5 WA State Department of Ecology. 2008. Manchester Environmental Laboratory Lab User's Manual. Accessed April 2014.
- 10.6 WA State Department of Ecology. 2011. Chemical Hygiene Plan and Hazardous Materials Management Plan. Olympia, WA.

Attachment 1. Lab Analysis & Tracking Plan, (example only)

Note: The Lab Analysis & Tracking Plan may look different due to different fields and requirements of the project(s) involved, but fields will be available for documentation and cross reference of each sample's collection and processing information.

2009 Lab Analysis & Tracking Plan for Fish Samples																													
7 digit MEL Work Order # for WSTMP: 1001015-(xx)																													
															jar size ->		2 oz	4 oz	4 oz	4 oz	4 oz								
															min amount of tissue needed per jar ->		5 g	40 g	40 g	40 g	up to 90 g								
sort	WSTMP Exp 09 (X)	WSTMP LT 09 (L)	Hg Trends Fish 09 (M)	Lab Dup MEL	Lab Dup Contract	MS/MSD	Sites	Specie	Collect date	# fish avail	# fish in comp	Hg	3 PCB, 3 DDT, lipids	Pest, PCB, PBDE, lipids	PCDD/Fs, lipids	Field ID (for multiple composites use suffix 1,2,3 to indicate groups A,B,C, respectively)	WSTMP MEL Lab # (# assigned during fish processing)	Hg Trends Field ID	Hg Trends MEL Lab #	Process date	Process batch for MEL *	aliquot per fish (g)	skin: off or on	Hg wt (g)	3 PCB, 3 DDT, lipids (g)	Pest, PCB, PBDE, lipids (g)	PCDD/F lipids (g)	Archive wt (g)	COMMENTS (Bold = WSTMP; unbold = Hg Trends)
X							Amber L (S of Cheney)	RBT	10/15/09	10	5	1		1	1	AMBERRBT	28	-	-	1/12/10	a	150	ON	17	-	90	88	90	Process 3,4,6,7,10. Tossed extra fish.
X							Black L (nr Colville)	TT	9/30/09	15	5	1		1		BLACKTT	08	-	-	1/6/10	a	90	ON	12	-	90	-	89	Process 1,4,6,8,11. Tossed extra fish.
X							Duck L, Ocean Shores	LMB	9/21/09	10	5	1		1	1	DUCKLMB	19	-	-	1/11/10	a	90	ON	30	-	90	90	90	Process 1,2,3,9,10. Tossed extra fish.
X							Duck L, Ocean Shores	YP	9/21/09	10	5	1		1		DUCKYP	18	-	-	1/11/10	a	21	ON	10	-	46	-	46	Process 1,2,3,6,9
X							Duck L, Ocean Shores	BC	9/21/09	6	5	1		1		DUCKBC	17	-	-	1/11/10	a	37	ON	35	-	90	-	59	Process 1,2,3,4,5
X		M					Failor L	LMB	9/22/09	13	5	1		1		FAILLMB	69	FLHGLMB 1-13	11-23	11/10/09	a	75	ON	28	-	84	-	90	Hg Process Individuals 1-13. WSTMP wants 1,2,3,4,5 as 5-fish composite. CF/MF processed earlier in season. See BS for more info.
X		M					Failor L	CTT	9/22/09	17	5	1		1		FAILCTT	70	FLHGCTT1 FLHGCTT2 FLHGCTT3	24 25 26	11/12/09	a	47	ON	33	-	50	50	50	FLHGCTT1 - Process 15, 16, 17. FLHGCTT2 - Process 10,11, 9, 8. FLHGCTT3 - Process 3, 4, 7. For FLHGCTT3 - WSTMP wants 3,4,7,2,5 as 5-fish composite. Spare PCDD/F, lipids jar stored with arcs. CF/MF processed earlier in season.
X							Leo L (nr Colville)	YP	10/1/09	10	5	1		1		LEOYP	29	-	-	1/12/10	a	37	ON	11	-	72	-	42	Process 1,2,3,5,9. Tossed extra fish.
X							Leo L (nr Colville)	PMP	10/1/09	12	5	1		1		LEOPMP	30	-	-	1/12/10	a	28-33	ON	16	-	74	-	67	Process 3,5,6,7,9. Tossed extra fish.
X							Leo L (nr Colville)	BC	10/1/09	10	5	1		1		LEOBC	31	-	-	1/12/10	a	40	ON	10	-	62	-	66	Process 3,6,7,8,9. Tossed extra fish.
X		M					Pierre L	SMB	9/29/09	10	5	1		1		PIERSMB	68	PRHGSMB 1-10	1-10	11/10/09	a	107	ON	29	-	81	84	82	Hg process Individuals 1-10. WSTMP wants 1,2,3,4,5 as 5-fish composite. Spare PCDD/F, lipids jar stored with arcs. CF/MF processed earlier in season.
X	L						Snake R, Central Ferry	CC	10/28/09	13	5	1		1	1	SRCFCC1	09	-	-	1/8/10	a	118	off	10	-	90	91	92	A- 3,8,11,12,13
X	L						Snake R, Central Ferry	CC	10/28/09	13	4	1	1	1		SRCFCC2	10	-	-	1/8/10	a	106	off	40	90	-	90	90	B- 4,6,9,10
X	L						Snake R, Central Ferry	CC	10/28/09	13	4	1	1	1		SRCFCC3	11	-	-	1/8/10	a	137	off	40	85	-	85	85	C- 1,2,5,7
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1		1	1	SRCFCCP1	63	-	-	1/21/10	a	150	ON	32	-	85	85	85	A- 3,9,10
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1	1			SRCFCCP2	64	-	-	1/22/10	a	140	ON	40	90	-	-	90	B- 1,2,8
X	L						Snake R, Central Ferry	CCP	10/28/09	11	3	1	1			SRCFCCP3	65	-	-	1/21/10	a	150	ON	30	85	-	-	85	C- 5,6,7
Notes:																													
a = All samples were sent to MEL together (one large batch) on 1/28/10.																													

Attachment 2. Processing Bench Sheet, (example only)

Note: The Bench Sheet used during lab processing may look different due to different fields and requirements of the processes involved, but fields will be available for documentation and cross reference of each sample's information.

2009 WSTMP Fish Field and Bench Processing Data																				Processing crew:				
Sort #1: Waterbody, Species, TL descending or ascending										Sort #2: Waterbody, Species, ECY Field ID														
Waterbody	ECY Field ID	Species	WDFW Field ID	WDFW DNA ID (CD10-)	DNA Taken Y/N ¹	Total Length (mm)	Weight (gm)	FCI	Collect Date	Collect Method	Process date	Fillet weight (gm)	L, R, or B fillet	Skin status On/Off	Sex M/F	Fish age	Scale card #	Scale #	otolith tray #	otolith cell #	Opercula or Spines taken Y/N	Comment	WSTMP LAR Field ID	WSTMP MEL Lab #
Whatcom Lk	1	SMB	-	652	Y	443	1420	1.63	10/6/09	ecy-G	12/3/09	203	L	ON	F	7	9	1	3	11	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	2	SMB	-	646	Y	372	764	1.48	10/6/09	ecy-G	12/3/09	136	L	ON	F	5	9	2	3	12	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	3	SMB	-	647	Y	375	790	1.50	10/6/09	ecy-G	12/3/09	124	L	ON	F	5	9	3	3	13	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	4	SMB	-	-	N	340	588	1.50	10/6/09	ecy-G	12/3/09	97	L	ON	M	4	9	4	3	14	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	5	SMB	-	648	Y	330	573	1.59	10/6/09	ecy-G	12/3/09	105	L	ON	M	4	9	5	3	15	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	6	SMB	-	649	Y	284	337	1.47	10/6/09	ecy-G	12/3/09	74	L	ON	M	3	10	6	3	16	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	7	SMB	-	650	Y	400	1166	1.82	10/7/09	ecy-G	12/3/09	215	L	ON	M	5	10	7	3	17	N	Do as indiv for HG Trend. Also do as 5 fish comp for WSTMP. All caught in SW end of Lk. See LTS for more details.	WHATSMB	12
Whatcom Lk	8	SMB	-	653	Y	340	565	1.44	10/7/09	ecy-G	12/3/09	119	L	ON	F	4	10	8	3	18	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	9	SMB	-	654	Y	306	411	1.43	10/7/09	ecy-G	12/3/09	85	L	ON	F	3	10	9	3	19	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Whatcom Lk	10	SMB	-	655	Y	285	314	1.36	10/7/09	ecy-G	12/3/09	71	L	ON	U	3	10	10	3	20	N	Do as indiv for HG Trend. All caught in SW end of Lk. See LTS for more details.	-	-
Notes:																								
1. In an effort to streamline Ecology's fish processing, DNA samples are only collected when requested by WDFW, USFWS, or stated in permit conditions. Individual project managers typically decide if timeframes and staffing allow for this extra processing step.																								

Attachment 3. Supplemental Bench Sheet for Processing Fillet and Carcass Tissue

Supplemental bench sheet for processing fillet and carcass tissue on selected samples.					
<p>The ratio of contaminant concentration in fillets to whole fish is needed to estimate concentrations of contaminants in fillet tissue when only whole-fish data are available. Determining this ratio requires a mass balance approach using concentration and weight data from fillets and the remaining carcass. To account for changes in weight of each fish during processing (e.g. weight loss from scale removal), the weight of each fish and its various tissues should be recorded at selected steps in the processing procedure. Record the following weights to nearest gram for each fish processed within a composite sample for fillet/carcass analysis. Remember that the composite of fillets and composite of carcass tissue from the same group of fish are treated as two separate samples - so clean equipment is needed for each sample.</p>					
Site					
Species					
Fish ID					
Field Weight (from field notes)					
Process Date					
Lab Weight (weigh fish prior to scale and slime removal)					
Whole fish after scales and DNA sample removed (ready to fillet)					
Fillet tissue ready for grinder (both fillets)					
Operculum					
Otoliths					
Spines					
Remaining carcass ready for grinder					
other					

Material Safety Data Sheet

Nitric acid, 20-70%

ACC# 16550

Section 1 - Chemical Product and Company Identification

MSDS Name: Nitric acid, 20-70%

Catalog Numbers: AC124660000, AC124660010, AC124660011, AC124660025, AC124660026, AC124665000, AC124665001, AC133620000, AC133620010, AC133620011, AC133620025, AC133620026, AC424000000, AC424000025, AC424000026, AC424000250, AC424005000, AC424005001, AC613205000, S71972, S719721, S71972MF, S71972SC, S756232, S76523, S93314, S93315, A198C-212, A198C4X-212, A200-212, A200-500, A200-612GAL, A200212LC, A200C-212, A200C212EA, A200C212LC, A200C4X-212, A200C4X212L, A200S-212, A200S-500, A200SI-212, A206C-212, A206C4X-212, A467-1, A467-2, A467-250, A467-500, A483-212, A509-212, A509-212LC, A509-500, A509SK-212, A509SK-212LC, M-281, MCC-030822

Synonyms: Azotic acid; Engraver's acid; Aqua fortis.

Company Identification:

Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410

For information, call: 201-796-7100

Emergency Number: 201-796-7100

For CHEMTREC assistance, call: 800-424-9300

For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7732-18-5	Water	30-80	231-791-2
7697-37-2	Nitric acid	20-70	231-714-2

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: clear to yellow liquid.

Danger! May be fatal if inhaled. Causes severe eye and skin burns. Causes severe respiratory and digestive tract burns. Strong oxidizer. Contact with other material may cause a fire. Acute pulmonary edema or chronic obstructive lung disease may occur from inhalation of the vapors of nitric acid. Corrosive to metal.

Target Organs: Lungs, eyes, skin, mucous membranes.

Potential Health Effects

Eye: Causes severe eye burns. Direct contact with liquid may cause blindness or permanent eye damage.

Skin: Causes skin burns. May cause deep, penetrating ulcers of the skin. Concentrated nitric acid dyes human skin yellow on contact.

Ingestion: May cause severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns. May cause perforation of the digestive tract. May cause systemic effects.

Inhalation: Effects may be delayed. Causes chemical burns to the respiratory tract. Inhalation may be fatal as a result of spasm, inflammation, edema of the larynx and bronchi, chemical pneumonitis and pulmonary edema. Aspiration may lead to pulmonary edema. May cause systemic effects. May cause acute pulmonary edema, asphyxia, chemical pneumonitis, and upper airway obstruction caused by edema. Depending on the conditions, the vapor or fumes of nitric acid may actually be a mixture of nitric acid and various oxides of nitrogen. The composition may vary with temperature, humidity, and contact with other organic materials.

Chronic: Exposure to high concentrations of nitric acid vapor may cause pneumonitis and pulmonary edema which may be fatal. Symptoms may or may not be delayed. Continued exposure to the vapor & mist of nitric acid may result in a chronic bronchitis, & more severe exposure results in a chemical pneumonitis. The vapor & mists of nitric acid may erode the teeth, particularly affecting the canines & incisors.

Section 4 - First Aid Measures

Eyes: Get medical aid immediately. Do NOT allow victim to rub eyes or keep eyes closed. Extensive irrigation with water is required (at least 30 minutes).

Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Destroy contaminated shoes.

Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.

Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Do NOT use mouth-to-mouth resuscitation. If breathing has ceased apply artificial respiration using oxygen and a suitable mechanical device such as a bag and a mask.

Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Strong oxidizer. Contact with other material may cause fire. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Use water spray to keep fire-exposed containers cool. May react with metal surfaces to form flammable and explosive hydrogen gas. Approach fire from upwind to avoid hazardous vapors and toxic decomposition products.

Extinguishing Media: Use extinguishing media most appropriate for the surrounding fire.

Flash Point: Not applicable.

Autoignition Temperature: Not available.

Explosion Limits, Lower: Not available.

Upper: Not available.

NFPA Rating: (estimated) Health: 4; Flammability: 0; Instability: 0; Special Hazard: OX

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.

Spills/Leaks: Avoid runoff into storm sewers and ditches which lead to waterways. Clean up spills immediately, observing precautions in the Protective Equipment section. Absorb spill using an absorbent, non-combustible material such as earth, sand, or vermiculite. Do not use combustible materials such as sawdust. Provide ventilation. Evacuate unnecessary personnel. Approach spill from upwind. Use water spray to cool and disperse vapors and protect personnel.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Do not breathe dust, vapor, mist, or gas. Do not get in eyes, on skin, or on clothing. Keep container tightly closed. Avoid contact with clothing and other combustible materials. Discard contaminated shoes. Do not use with metal spatula or other metal items. Use only with adequate ventilation or respiratory protection.

Storage: Do not store near combustible materials. Do not store in direct sunlight. Keep container closed when not in use. Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from metals. Store away from alkalis. Separate from organic materials. Inspect periodically for damage or evidence of leaks or corrosion.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. Use a corrosion-resistant ventilation system.

Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Water	none listed	none listed	none listed
Nitric acid	2 ppm TWA; 4 ppm STEL	2 ppm TWA; 5 mg/m ³ TWA 25 ppm IDLH	2 ppm TWA; 5 mg/m ³ TWA

OSHA Vacated PELs: Water: No OSHA Vacated PELs are listed for this chemical. Nitric acid: 2 ppm TWA; 5 mg/m³ TWA

Personal Protective Equipment

Eyes: Wear chemical splash goggles and face shield.

Skin: Wear appropriate gloves to prevent skin exposure.

Clothing: Wear appropriate clothing to prevent skin exposure.

Respirators: Wear a NIOSH/MSHA or European Standard EN 149 approved full-facepiece airline respirator in the positive pressure mode with emergency escape provisions.

Section 9 - Physical and Chemical Properties

Physical State: Liquid

Appearance: clear to yellow

Odor: strong odor - acrid odor - suffocating odor

pH: 1.0 (0.1M soln)

Vapor Pressure: 7.1 mm Hg @ 20 deg C (70% acid)

Vapor Density: 2.17 (air=1)

Evaporation Rate: Not available.

Viscosity: 0.761 cps @ 25 deg C

Boiling Point: 86 deg C

Freezing/Melting Point: -42 deg C

Decomposition Temperature: Not available.

Solubility: Soluble in water.

Specific Gravity/Density: 1.4

Molecular Formula: HNO₃

Molecular Weight: 63.01

Section 10 - Stability and Reactivity

Chemical Stability: Stable. Decomposes when in contact with air, light, or organic matter. The yellow color is due to release of nitrogen dioxide on exposure to light.

Conditions to Avoid: High temperatures, light, confined spaces.

Incompatibilities with Other Materials: Metals, reducing agents, strong bases, acetic acid, alcohols, acetone, aniline, hydrogen sulfide, metal powders, carbides, aldehydes, organic solvents, combustible materials, chromic acid, flammable liquids, cyanides, sulfides, Incompatible with many substances.

Hazardous Decomposition Products: Nitrogen oxides.

Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:

CAS# 7732-18-5: ZC0110000

CAS# 7697-37-2: QU5775000; QU5900000

LD50/LC50:

CAS# 7732-18-5:

Oral, rat: LD50 = >90 mL/kg;

CAS# 7697-37-2:

Inhalation, rat: LC50 = 260 mg/m³/30M;

Inhalation, rat: LC50 = 130 mg/m³/4H;

Inhalation, rat: LC50 = 67 ppm(NO₂)/4H;

Carcinogenicity:

CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

CAS# 7697-37-2: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No information found

Teratogenicity: No information found

Reproductive Effects: No information found

Mutagenicity: No information found

Neurotoxicity: No information found

Other Studies:

Section 12 - Ecological Information

Ecotoxicity: No data available. No information available.

Environmental: Terrestrial: During transport through the soil, nitric acid will dissolve some of the soil material, in particular, the carbonate based materials. The acid will be neutralized to some degree with adsorption of the proton also occurring on clay materials. However, significant amounts of acid are expected to remain for transport down toward the ground water table. Upon reaching the ground water table, the acid will continue to move, now in the direction of the ground water flow.

Physical: No information available.

Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.

RCRA P-Series: None listed.

RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	NITRIC ACID	NITRIC ACID
Hazard Class:	8	8(9.2)
UN Number:	UN2031	UN2031
Packing Group:	II	II

Section 15 - Regulatory Information

US FEDERAL

TSCA

CAS# 7732-18-5 is listed on the TSCA inventory.

CAS# 7697-37-2 is listed on the TSCA inventory.

Health & Safety Reporting List

None of the chemicals are on the Health & Safety Reporting List.

Chemical Test Rules

None of the chemicals in this product are under a Chemical Test Rule.

Section 12b

None of the chemicals are listed under TSCA Section 12b.

TSCA Significant New Use Rule

None of the chemicals in this material have a SNUR under TSCA.

CERCLA Hazardous Substances and corresponding RQs

CAS# 7697-37-2: 1000 lb final RQ; 454 kg final RQ

SARA Section 302 Extremely Hazardous Substances

CAS# 7697-37-2: 1000 lb TPQ

SARA Codes

CAS # 7697-37-2: immediate, delayed, fire.

Section 313

This material contains Nitric acid (CAS# 7697-37-2, 20-70%), which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.

Clean Air Act:

This material does not contain any hazardous air pollutants.

This material does not contain any Class 1 Ozone depletors.

This material does not contain any Class 2 Ozone depletors.

Clean Water Act:

CAS# 7697-37-2 is listed as a Hazardous Substance under the CWA.

None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.

OSHA:

CAS# 7697-37-2 is considered highly hazardous by OSHA.

STATE

CAS# 7732-18-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

CAS# 7697-37-2 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

C

Risk Phrases:

R 35 Causes severe burns.

Safety Phrases:

S 23 Do not inhale gas/fumes/vapour/spray.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36 Wear suitable protective clothing.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

WGK (Water Danger/Protection)

CAS# 7732-18-5: No information available.

CAS# 7697-37-2: 1

Canada - DSL/NDSL

CAS# 7732-18-5 is listed on Canada's DSL List.

CAS# 7697-37-2 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of E, C, D1A.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

CAS# 7697-37-2 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 9/30/1998

Revision #13 Date: 12/14/2004

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.



Burdick & Jackson

Material Safety Data Sheet

Acetone

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: Acetone

OTHER/GENERIC NAMES: Acetone NF, 2-Propanone, Diethyl Ketone Dimethylketal, Dimethylformaldehyde Pyroacetic acid, Pyroacetic ether

PRODUCT USE: Solvent

MANUFACTURER: Honeywell, Burdick & Jackson
1953 South Harvey Street
Muskegon, MI 49442

FOR MORE INFORMATION CALL:
(Monday-Friday, 8:00am-5:00pm Eastern Time)
1-800-368-0050

IN CASE OF EMERGENCY CALL:
(24 Hours/Day, 7 Days/Week)
1-800-707-4555 (Honeywell -Domestic)
602-365-4980 (Honeywell - International)
For Transportation Emergencies:
1-800-424-9300 (CHEMTREC - Domestic)
703-527-3887 (CHEMTREC - International)

2. COMPOSITION/INFORMATION ON INGREDIENTS

<u>INGREDIENT NAME</u>	<u>CAS NUMBER</u>	<u>WEIGHT %</u>
Acetone	67-64-1	100

Additional material names not listed above may also appear in Section 15 toward the end of the MSDS. These materials may exist in trace amounts at the part-per-million level, and may be listed for local "Right-To-Know" compliance and for other regulatory reasons.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW:

Flammable liquid and vapor. Vapor may cause flash fire. Clear, colorless liquid that causes skin, eye and respiratory tract irritation. Harmful if swallowed or inhaled.

POTENTIAL HEALTH HAZARDS

SKIN: Repeated and/or prolonged exposures to the skin may result in itching, redness, drying, scaling, and peeling.

EYES: Vapors are irritating to the eyes. Liquid contact produces intense stinging and burning sensations.

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Acetone

INHALATION: Exposure can cause respiratory tract and throat irritation, headaches, shortness of breath and symptoms similar to intoxication. Overexposure can produce severe central nervous system depression, coma and respiratory failure.

INGESTION: Ingestion causes a burning sensation in the mouth, throat and stomach followed by nausea and vomiting. Small amounts aspirated into the lungs can cause chemical pneumonia, lung injury and death.

DELAYED EFFECTS: None known.

Ingredients that are found on one of the OSHA designated carcinogen lists are listed below.

<u>INGREDIENT NAME</u>	<u>NTP STATUS</u>	<u>IARC STATUS</u>	<u>OSHA LIST</u>
No ingredients listed in this section			

4. FIRST AID MEASURES

SKIN: Immediately rinse affected area with plenty of water for 15 minutes. Get medical attention as needed for irritation or any other symptoms. Launder contaminated clothing before reuse.

EYES: Immediately flush eyes with large amounts of water for at least 15 minutes. Get immediate medical attention.

INHALATION: Remove from exposure area to fresh air. If breathing is difficult, give oxygen provided a qualified operator is available. If breathing has stopped, apply artificial respiration. Get immediate medical attention.

INGESTION: **Aspiration hazard.** If conscious, rinse mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Get immediate medical attention.

ADVICE TO PHYSICIAN: A. Treatment of severe systemic intoxication (narcoosis) from either vapor exposure or ingestion is primarily supportive. Acetone has minimal toxicity on other organ systems and if the victim can be supported through the period of central nervous system depression and respiratory failure, the prognosis is good.

(1) Following recent ingestion, acetone may be removed by gastric lavage. Emesis is not recommended.

Activated charcoal is recommended.

(2) Mechanically assisted ventilation may be necessary.

(3) Treat symptomatically and monitor blood glucose.

B. Eye exposures usually do not require any specific treatment if liquid acetone is promptly washed out of eyes. If exposure was prolonged, some initial corneal damage may be present. It is advisable for these individuals to be seen by an ophthalmologist.

Burdick & Jackson**MATERIAL SAFETY DATA SHEET**Acetone

5. FIRE FIGHTING MEASURES

FLAMMABLE PROPERTIES

FLASH POINT:	-4°F (-20°C)
FLASH POINT METHOD:	Closed Cup
AUTOIGNITION TEMPERATURE:	869°F (465°C)
UPPER FLAMMABLE LIMIT (volume % in air):	13% v/v
LOWER FLAMMABLE LIMIT (volume % in air):	2.5%v/v
FLAME PROPAGATION RATE (solids):	Not Applicable
OSHA FLAMMABILITY CLASS:	IB

EXTINGUISHING MEDIA:

Dry chemical, foam, or carbon dioxide. Water spray may be used to cool fire exposed containers.

UNUSUAL FIRE AND EXPLOSION HAZARDS:

Extremely flammable. Vapors form explosive mixtures with air. Vapors may spread long distances and ignite. Dangerous when exposed to heat, sparks, flame or oxidants. Sealed containers may rupture when heated.

SPECIAL FIRE FIGHTING PRECAUTIONS/INSTRUCTIONS:

Handle as a very flammable liquid. Water will not be effective in extinguishing a fire. Use water spray to cool fire-exposed containers and to reduce rate of burning, taking care not to spread the fire. Heat will build pressure and rupture closed storage containers. Vapors can travel to distant ignition source and flash back. Wear NIOSH approved self-contained breathing apparatus, and full protective clothing. Do not release runoff from fire control measures into waterways or sewers.

6. ACCIDENTAL RELEASE MEASURES

IN CASES OF SPILL OR OTHER RELEASE: Always wear recommended personal protective equipment.)

Eliminate sources of ignition. Isolate the spill area. Use non-sparking tools and equipment. Stop leak in a safe and practical manner. (If leak cannot be stopped easily and safely, advise trained emergency response personnel of the situation.) Contain and recover liquid when possible. Absorb small spills with an inert material and place in an approved chemical waste container. For large spills, dike up with inert material and transfer into same container. Do not allow to enter into drains or waterways.

Spills and releases may have to be reported to Federal and/or local authorities. See Section 15 regarding reporting requirements.

7. HANDLING AND STORAGE

NORMAL HANDLING: (Always wear recommended personal protective equipment.)

Ground containers for transfer of contents. Keep away from heat, sparks, open flames and ignition sources. Do not get in eyes, on skin or clothing. Use with adequate ventilation. No smoking in areas of use. Wash contaminated clothing and protective equipment before reuse.

MSDS Number: B&J 0010
Current Issue Date: June 21, 2002

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Burdick & Jackson**MATERIAL SAFETY DATA SHEET**Acetone

STORAGE RECOMMENDATIONS:

Store in an area designed for storage of flammable liquids. (OSHA 29 CFR 1910.106)
Protect from temperature extremes and sunlight, and store away from incompatible substances and in accordance with 29 CFR 1910.106. Avoid acids, bases, oxidizers, explosives, nitrogen-fluorine compounds, sulfites, perchlorates, reducing agents and plastics. Empty containers may contain product residue and/or vapors. Label warnings apply to empty containers that have not been cleaned.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS:

Provide general or local exhaust ventilation systems to maintain airborne concentrations below exposure limits. Local exhaust ventilation is preferred because it prevents contaminant dispersion into the work area by controlling it at its source.

PERSONAL PROTECTIVE EQUIPMENT**SKIN PROTECTION:**

Wear impervious gloves, boots and clothing suitable to prevent skin contact. Inspect for signs of degradation before each use. Replace as needed. Safety-toed shoes should be worn when handling drums.

EYE PROTECTION:

Wear safety glasses with non-perforated sideshields for normal handling. Goggles or a full-face shield may be necessary depending on quantity of material and conditions of use. Contact lens should not be worn when working with this chemical.

RESPIRATORY PROTECTION:

Not required for properly ventilated areas. If there is potential for inhalation of vapor or mist, use an appropriate NIOSH approved respirator. Warning! Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

The respirator must be selected based on contamination levels and use conditions found in the workplace. Use conditions must not exceed the working limits of the respirator. The respirator must be approved by the National Institute for Occupational Safety and Health (NIOSH) and used in accordance with Occupational Safety and Health Administration (OSHA) 29 CFR 1910.134.

ADDITIONAL RECOMMENDATIONS:

Provide eyewash station and safety showers convenient to work areas. Do not eat, drink or smoke in work areas.

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Acetone

EXPOSURE GUIDELINES

<u>INGREDIENT NAME</u>	<u>ACGIH TLY</u>	<u>OSHA Z-1 PEL</u>	<u>OTHER LIMIT</u>
Acetone	500 ppm TWA (8-hr. exposure limit) 750 ppm: 15 min. STEL	1000 ppm	NIOSH REL.: 250 ppm 10 hr day/40 hr week. NIOSH IDLH: 2500 ppm.

OTHER EXPOSURE LIMITS FOR POTENTIAL DECOMPOSITION PRODUCTS:

None known

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE:	Clear, colorless
PHYSICAL STATE:	Liquid
MOLECULAR WEIGHT:	58.08
CHEMICAL FORMULA:	C ₃ H ₆ O
ODOR:	Sweet mint-like odor detectable at 20 ppm
SPECIFIC GRAVITY (water = 1.0):	0.79
SOLUBILITY IN WATER (weight %):	Complete
pH:	Not Applicable
BOILING POINT:	133°F (56°C)
MELTING POINT:	-94.8°C
VAPOR PRESSURE:	180 mm Hg at 20°C
VAPOR DENSITY (air = 1.0):	2.0
EVAPORATION RATE:	12 COMPARED TO: Butyl Acetate = 1
% VOLATILES:	100
FLASH POINT:	-4°F (-20°C)

(Flash point method and additional flammability data are found in Section 5.)

10. STABILITY AND REACTIVITY

NORMALLY STABLE? (CONDITIONS TO AVOID):

Product is stable at ambient room temperature in closed containers. Keep away from heat, sparks and flame.

INCOMPATIBILITIES:

Acids and oxidizers.

HAZARDOUS DECOMPOSITION PRODUCTS:

Complete combustion results in the formation of carbon dioxide and water vapor. Incomplete combustion can produce carbon monoxide and other toxic oxides of carbon.

HAZARDOUS POLYMERIZATION:

Will not occur.

Burdick & Jackson**MATERIAL SAFETY DATA SHEET**

Acetone

11. TOXICOLOGICAL INFORMATION**IMMEDIATE (ACUTE) EFFECTS:**

Oral (rat) LD₅₀ = 5800 mg/kg
Oral (mouse) LD₅₀ = 3000 mg/kg
Oral (rabbit) LD₅₀ = 5340 mg/kg
Inhalation (rat) LC₅₀ = 32000 ppm, 4-hr
Dermal (guinea pig) LD₅₀ =>9400 µL/kg
Skin Irritation (rabbit) = Mild, 500 mg/24 hr
Eye Irritation (rabbit) = moderate to severe, 20 mg, damage generally limited to corneal epithelium and is reversible.

DELAYED (SUBCHRONIC AND CHRONIC) EFFECTS:

8-Week Inhalation Toxicity Study (rat): 19,000 ppm acetone 5days/week for 8 weeks produced no signs of toxicity other than slightly reduced weight gain compared to controls.
90-Day Oral Toxicity Study (rat): The no-observed effect level is 100 mg/kg/day and the low-observed effect level is 500 mg/kg/day based on increased liver and kidney weights and nephrotoxicity.

OTHER DATA:

Ames Assay (S. typhimurium): Negative
Chromosome Aberrations and Sister Chromatid Exchange Assays: Negative
Point Mutation in Mouse Lymphoma Cells: Negative
DNA Cell-binding Assay: Negative

12. ECOLOGICAL INFORMATION

96-Hr LC₅₀ (rainbow trout) = 5,540 mg/L, 12° C
24- to 48-Hr LC₅₀ (Daphnia magna) = 10 mg/L
96-Hr LC₅₀ (bluegill sunfish) = 8300 mg/L

Octanol/Water Partition Coefficient: 0.58
Biological Oxygen Demand: 122%, 5 days
Bioconcentration Factor (BCF): 1, suggesting bioconcentration in aquatic organisms is low; calculated using an experimental
log Kow of -0.24.

13. DISPOSAL CONSIDERATIONS**RCRA**

Is the unused product a RCRA hazardous waste if discarded? Yes
If yes, the RCRA ID number (USEPA Hazardous Waste Code) is: U002 and D001

OTHER DISPOSAL CONSIDERATIONS:

Whatever cannot be saved for recovery or recycling should be handled as hazardous waste and sent to a RCRA approved incinerator or RCRA approved waste facility. Dispose of container and unused contents in accordance with federal, state and local requirements.

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Acetone

The information offered here is for the product as shipped. Use and/or alterations to the product such as mixing with other materials may significantly change the characteristics of the material and alter the RCRA classification and the proper disposal method.

14. TRANSPORT INFORMATION

Proper DOT Shipping Description: Acetone, 3, UN 1090, II.

Reportable Quantity (RQ): 5000 lbs (2270 kg).

Label(s) Required: Class 3, Flammable Liquid.

Emergency Response Guidebook (2000 Edition): Guide No. 127.

For additional information on shipping regulations affecting this material, contact the information number found in Section 1.

15. REGULATORY INFORMATION

TOXIC SUBSTANCES CONTROL ACT (TSCA)

TSCA INVENTORY STATUS: Acetone is listed on TSCA inventory.

OTHER TSCA ISSUES: TSCA 4(a) Final Test Rules & Testing Consent Orders.
TSCA 8(a) Inventory Update Rule. (1998 EPA form U Instructions, App.A)
TSCA 12(b) Export Notification. One-time Export Notification. Notice required only for first export or intended export to a particular country. [40 CFR 707.65(a)(2)(ii)]

SARA TITLE III/CERCLA

"Reportable Quantities" (RQs) and/or "Threshold Planning Quantities" (TPQs) exist for the following ingredients.

<u>INGREDIENT NAME</u>	<u>SARA/CERCLA RQ (lb)</u>	<u>SARA EHS TPQ (lb)</u>
Acetone	5000	None

Spills or releases resulting in the loss of any ingredient at or above its RQ requires immediate notification to the National Response Center [(800) 424-8802] and to your Local Emergency Planning Committee.

SECTION 311 HAZARD CLASS: Immediate, Fire.

SARA 313 TOXIC CHEMICALS:

The following ingredients are SARA 313 "Toxic Chemicals". CAS numbers and weight percents are found in Section 2.

<u>INGREDIENT NAME</u>	<u>COMMENT</u>
No ingredients listed in this section	

STATE RIGHT-TO-KNOW

In addition to the ingredients found in Section 2, the following are listed for state right-to-know purposes.

<u>INGREDIENT NAME</u>	<u>WEIGHT %</u>	<u>COMMENT</u>
------------------------	-----------------	----------------

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Acetone

No ingredients listed in this section.

None.

ADDITIONAL REGULATORY INFORMATION:

Acetone is a DEA Listed Precursor and Essential Chemical (List II) subject to certain import, export recordkeeping and reporting requirements. 21 CFR 1310.04 (f),(g).

Acetone is a Volatile organic compound (VOC) with negligible photochemical reactivity and thus excluded from the definition of volatile organic compounds for the purposes of preparing State implementation plans to attain the national ambient air quality standards for ozone under title I of the Clean Air Act, 40 CFR 51.100(s)

WHMIS CLASSIFICATION (CANADA):

Class B, Division 2.

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by the Controlled Products Regulations.

FOREIGN INVENTORY STATUS:

Acetone is listed on the following inventories:

- Australian.
- Canadian DSL.
- Chinese.
- EDNECS.
- Japanese (ENCS).
- Korean.
- Philippine (PICCS).

16. OTHER INFORMATION

CURRENT ISSUE DATE: June 21, 2002.

PREVIOUS ISSUE DATE: June, 2000.

CHANGES TO MSDS FROM PREVIOUS ISSUE DATE ARE DUE TO THE FOLLOWING:

MSDS Number: B&J 0010
Current Issue Date: June 21, 2002

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MATERIAL SAFETY DATA SHEET

Acetone

Amended or modified the following:

- Hazards Identification, Section 3.
- First Aid Measures, Section 4.
- Special Fire Fighting Precautions/Instructions, Section 5.
- Accidental Release Measures, Section 6.
- Personal Protective Equipment & Exposure Guidelines, Section 8.
- Toxicological Information, Section 11.
- Ecological Information, Section 12.
- Transport Information, Section 14.
- Additional Regulatory Information & Foreign Inventory Status, Section 15.

OTHER INFORMATION: **NFPA Classification**
Health: 1
Flammability: 3
Reactivity: 0

Honeywell

Burdick & Jackson

Material Safety Data Sheet

Hexane

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME: Hexane
OTHER/GENERIC NAMES: n-Hexane,
Hexyl hydride
PRODUCT USE: Solvent
MANUFACTURER: Honeywell, Burdick & Jackson
1953 South Harvey Street
Muskegon, MI 49442

FOR MORE INFORMATION CALL:
(Monday-Friday, 8:00am-5:00pm Eastern Time)
1-800-368-0050

IN CASE OF EMERGENCY CALL:
(24 Hours/Day, 7 Days/Week)
1-800-707-4555 (Honeywell -Domestic)
602-365-4980 (Honeywell - International)
For Transportation Emergencies:
1-800-424-9300 (CHEMTREC - Domestic)
703-527-3887 (CHEMTREC - International)

2. COMPOSITION/INFORMATION ON INGREDIENTS

<u>INGREDIENT NAME</u>	<u>CAS NUMBER</u>	<u>WEIGHT %</u>
Hexane	110-51-3	~100

Trace impurities and additional material names not listed above may also appear in Section 15 toward the end of the MSDS. These materials may be listed for local "Right-To-Know" compliance and for other reasons.

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW: Flammable liquid and vapor. Harmful if swallowed, inhaled or absorbed through the skin. Causes skin, eye and respiratory tract irritation. Central nervous system depressant.

POTENTIAL HEALTH HAZARDS

SKIN: Can cause dermatitis through defatting of the skin. May be absorbed through the skin with possible systemic effects.

EYES: Irritant. Redness and itching may occur.

Burdick & Jackson**MATERIAL SAFETY DATA SHEET**

Hexane

- INHALATION:** Can cause lightheadedness, giddiness, headache, extremity numbness, central nervous system depression and respiratory tract irritation.
- INGESTION:** Ingestion can cause same effects as inhalation plus gastrointestinal tract discomfort. Aspiration into the lungs can cause chemical pneumonia and lung damage.
- DELAYED EFFECTS:** Effects of CNS depression may linger for hours after exposure. Peripheral neuropathies can occur with long-term exposure.

Ingredients found on one of the OSHA designated carcinogen lists are listed below.

<u>INGREDIENT NAME</u>	<u>NTP STATUS</u>	<u>IARC STATUS</u>	<u>OSHA LIST</u>
No ingredients listed in this section.			

4. FIRST AID MEASURES

- SKIN:** Wash with soap and water and flush with water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention for irritation or any other symptom. Launder contaminated clothing and clean shoes before reuse.
- EYES:** Immediately flush eyes with plenty of water for at least 15 minutes. Get immediate medical attention.
- INHALATION:** Remove from exposure area to fresh air. If breathing is difficult, give oxygen provided a qualified operator is available. If breathing has stopped, apply artificial respiration. Get immediate medical attention.
- INGESTION:** **Aspiration hazard.** If conscious, rinse mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Get immediate medical attention.
- ADVICE TO PHYSICIAN:** Treat supportively and symptomatically.

5. FIRE FIGHTING MEASURES**FLAMMABLE PROPERTIES**

FLASH POINT: -7°F (-22°C)
FLASH POINT METHOD: Closed Cup
AUTOIGNITION TEMPERATURE: 437°F
UPPER FLAMMABLE LIMIT (volume % in air): 7.5%
LOWER FLAMMABLE LIMIT (volume % in air): 1.1%
FLAME PROPAGATION RATE (solids): Not Applicable
OSHA FLAMMABILITY CLASS: II

EXTINGUISHING MEDIA:

Carbon dioxide, dry chemical, regular foam or water spray.

UNUSUAL FIRE AND EXPLOSION HAZARDS:

MSDS Number: B&J 0217
Current Issue Date: June 21, 2002.

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MATERIAL SAFETY DATA SHEET

Hexane

Fire hazard and moderate explosion hazard when exposed to an ignition source. Containers can pressurize and rupture during fire conditions.

SPECIAL FIRE FIGHTING PRECAUTIONS/INSTRUCTIONS:

Do not release runoff from fire control methods to sewers or waterways. Fire may produce toxic thermal decomposition products. Wear a NIOSH approved self-contained breathing apparatus (SCBA) with a full facepiece operated in pressure-demand or positive-pressure mode.

6. ACCIDENTAL RELEASE MEASURES

IN CASE OF SPILL OR OTHER RELEASE: (Always wear recommended personal protective equipment.)

Eliminate sources of ignition. Isolate the spill area. Use non-sparking tools and equipment. Stop leak in a safe and practical manner. (If leak cannot be stopped easily and safely, advise trained emergency response personnel of the situation.) Contain and recover liquid when possible. Absorb small spills with an inert material and place in an approved chemical waste container. For large spills, dike up with inert material and transfer into same container. Do not allow to enter into drains or waterways.

Spills and releases may have to be reported to Federal and/or local authorities. See Section 15 regarding reporting requirements.

7. HANDLING AND STORAGE

NORMAL HANDLING: (Always wear recommended personal protective equipment.)

Use with adequate ventilation. Avoid contact with skin, eyes and clothing. Do not breathe vapors. Flammable liquid and vapors. Keep away from heat, sparks and flame. Electrically ground all handling equipment. Keep container closed when not in use.

STORAGE RECOMMENDATIONS:

Store in an area suitable for flammable liquids (OSHA 29 CFR 1910.106). Store away from ignition sources, temperature extremes, direct sunlight and incompatible substances. **Empty containers may contain product residue and/or vapors. Label warnings apply to empty containers that have not been cleaned.**

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS:

Provide general or local exhaust ventilation systems. Local exhaust ventilation is preferred because it prevents contaminant dispersion into the work area by controlling it at its source.

PERSONAL PROTECTIVE EQUIPMENT

SKIN PROTECTION:

Wear protective gloves, boots and clothing suitable to prevent skin contact. Nitrile, Polyvinyl Alcohol (PVA) and Neoprene are suitable materials of construction. Inspect for signs of degradation after each use. Replace as needed.

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Hexane

EYE PROTECTION:

Wear safety glasses or chemical safety goggles, per OSHA eye and face protection regulations (29 CFR 1910.133). Contact lenses are not eye protective devices. Appropriate eye protection must be worn instead of, or in conjunction with contact lenses.

RESPIRATORY PROTECTION:

Not required for properly ventilated areas. If there is potential for inhalation of vapor or mist, use an appropriate NIOSH approved respirator. Warning! Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

The respirator must be selected based on contamination levels and use conditions found in the workplace. Use conditions must not exceed the working limits of the respirator. The respirator must be approved by the National Institute for Occupational Safety and Health (NIOSH) and used in accordance with Occupational Safety and Health Administration (OSHA) 29 CFR 1910.134.

ADDITIONAL RECOMMENDATIONS:

Make emergency eyewash stations and washing facilities available in work area.
Separate contaminated work clothes from street clothes. Launder before reuse. Remove this material from your shoes and clean personal protective equipment. Never eat, drink, or smoke in work areas. Practice good personal hygiene after using this material.

EXPOSURE GUIDELINES

<u>INGREDIENT NAME</u>	<u>ACGIH TLV</u>	<u>OSHA Z-1 PEL</u>	<u>OTHER LIMIT</u>
Hexane	50 ppm (skin) TWA 8-hr. exposure limit.	500 ppm	NIOSH REL: 50 ppm 10 hr day/40 hr week NIOSH IDLH: 1100 ppm.

OTHER EXPOSURE LIMITS FOR POTENTIAL DECOMPOSITION PRODUCTS:

None

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE:	Clear, Colorless	
PHYSICAL STATE:	Liquid	
MOLECULAR WEIGHT:	86.18	
CHEMICAL FORMULA:	CH ₃ (CH ₂) ₄ CH ₃	
ODOR:	Mild gasoline like odor. Threshold: Not listed	
SPECIFIC GRAVITY (water = 1.0):	0.659	
SOLUBILITY IN WATER (weight %):	0.01-4% @ 68°F (20°C)	
pH:	Not Applicable	
BOILING POINT:	155.7°F (68.7°C)	
MELTING POINT:	-139°F (-95°C)	
VAPOR PRESSURE:	124mm Hg @ 68°F (20°C)	
VAPOR DENSITY (air = 1.0):	3.0	
EVAPORATION RATE:	~16	COMPARED TO: Butyl Acetate = 1
% VOLATILES:	100%	
FLASH POINT:	-7.6°F (-22°C)	

(Flash point method and additional flammability data are found in Section 5.)

Burdick & Jackson**MATERIAL SAFETY DATA SHEET**

Hexane

10. STABILITY AND REACTIVITY**NORMALLY STABLE? (CONDITIONS TO AVOID):**

Stable at room temperature in closed containers under normal storage and handling conditions.

INCOMPATIBILITIES:

Strong oxidizers

CONDITIONS TO AVOID:

Heat, sparks, flame, incompatible materials.

HAZARDOUS DECOMPOSITION PRODUCTS:

Incomplete combustion can produce toxic fumes of carbon monoxide.

HAZARDOUS POLYMERIZATION:

Not expected to occur.

11. TOXICOLOGICAL INFORMATION**IMMEDIATE (ACUTE) EFFECTS:**Oral LD₅₀ (rat): 24 mL (juvenile); 45 mL (adult)Oral LD₅₀ (rat): 25 gm/kg.Inhalation LC₅₀ (rat): 48000 ppm/< 4-hrIntraperitoneal LD₅₀ (rat): 9,100 mg/kg.

Eye Irritation (rabbit): Mild, 10 mg

DELAYED (SUBCHRONIC AND CHRONIC) EFFECTS:

In humans, chronic exposure to an average air concentration of 450-650 ppm for as little as 2 months may result in peripheral neuropathy, characterized by muscular weakness, loss of sensation, and impaired gait.

In Sprague-Dawley rats, chronic exposure to n-hexane at 5000 ppm, 9 hrs/day, 5 days/week for 14 weeks or to 2500 ppm 10 hrs/day, 6 days/week for 30 weeks or produced pathological alterations characterized by giant axonal degeneration, and paranodal and internodal swellings of axons. A no-observed-adverse-effect level (NOAEL) of 1500 ppm was identified for pathological alterations of the peripheral nerves.

In Wistar rats, chronic exposure to n-hexane at 1200 or 3000 ppm, 12 hrs/day, 7 days/week for 16 weeks caused degeneration of peripheral nerves characterized by paranodal swellings and demyelination and remyelination in the myelinated nerve fibers. A NOAEL of 500 ppm was identified.

OTHER DATA:

In a 2-generation reproductive study in rats exposed to airborne concentrations of n-hexane up to 9000 ppm for 6 hrs/day, 5 or 7 days/week, mating, fertility, litter size, and postnatal survival were not significantly affected, although reduced weight gains were seen in the high dose group.

Burdick & Jackson**MATERIAL SAFETY DATA SHEET****Hexane**

In male rats, n-hexane at 1000 ppm can cause testicular damage when exposures are nearly continuous (18 hrs/day, 7 days/week for 61 days) and testicular tumors when exposures are repeated for nearly a life time (4 hrs/day for 59 weeks, intermittent).

Fetotoxicity in rats was produced when a concentration of 5000 ppm n-hexane was inhaled for 20 hrs/day on days 6 to 19 of pregnancy. n-Hexane has been demonstrated not to be teratogenic in rats or mice.

12. ECOLOGICAL INFORMATION

96h LC₅₀ (young Coho salmon) = 100 mg/L
24 h LC₅₀ (Goldfish) = 4 mg/l

13. DISPOSAL CONSIDERATIONS**RCRA**

Is the unused product a RCRA hazardous waste if discarded? Yes

If yes, the RCRA ID number (USEPA Hazardous Waste Code) is: D001

OTHER DISPOSAL CONSIDERATIONS:

Whatever cannot be saved for recovery or recycling should be handled as hazardous waste and sent to a RCRA approved incinerator or RCRA approved waste facility. Dispose of container and unused contents in accordance with federal, state and local requirements.

The information offered here is for the product as shipped. Use and/or alterations to the product such as mixing with other materials may significantly change the characteristics of the material and alter the RCA classification and the proper disposal.

14. TRANSPORT INFORMATION

Proper DOT Shipping Description: Hexanes, 3, UN 1208, II.

Reportable Quantity (RQ): Hexane = 5000 lbs (2270 kg).

Label(s) Required: Class 3, Flammable Liquid.

Emergency Response Guidebook (2000 Edition): Guide No. 128.

For additional information on shipping regulations affecting this material, contact the information number found in Section 1.

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Hexane

15. REGULATORY INFORMATION

TOXIC SUBSTANCES CONTROL ACT (TSCA)

TSCA INVENTORY STATUS: Listed on TSCA inventory.

OTHER TSCA ISSUES: None.

SARA TITLE III/CERCLA

"Reportable Quantities" (RQs) and/or "Threshold Planning Quantities" (TPQs) exist for the following ingredients.

<u>INGREDIENT NAME</u>	<u>SARA/CERCLA RQ (lb)</u>	<u>SARA EHS TPO (lb)</u>
Hexane	5000	None

Spills or releases resulting in the loss of any ingredient at or above its RQ requires immediate notification to the National Response Center [(800) 424-8802] and to your Local Emergency Planning Committee.

SECTION 311 HAZARD CLASS: Immediate, Delayed, Fire

SARA 313 TOXIC CHEMICALS:

The following ingredients are SARA 313 "Toxic Chemicals". CAS numbers and weight percents are found in Section 2.

<u>INGREDIENT NAME</u>	<u>COMMENT</u>
n-Hexane [110-54-3]	De Minimis Concentration is 1.0%

STATE RIGHT-TO-KNOW

In addition to the ingredients found in Section 2, the following are listed for state right-to-know purposes.

<u>INGREDIENT NAME</u>	<u>WEIGHT %</u>	<u>COMMENT</u>
No ingredients listed in this section.		

ADDITIONAL REGULATORY INFORMATION:

California Proposition 65 Label Statement

Hexane may contain Benzene in trace amounts (? 10 ppm, which is listed on one of the California Proposition 65 lists; therefore, the following statement has been placed on the product label:

"Warning: This product contains a chemical known to the State of California to cause cancer."

Burdick & Jackson

MATERIAL SAFETY DATA SHEET

Hexane

WHMIS CLASSIFICATION (CANADA):

Class B, Division 2

FOREIGN INVENTORY STATUS:

Hexane is listed on the following inventories:

- Australian.
- Canadian DSL.
- Chinese.
- EINECS.
- Japanese (ENCS).
- Korean.
- Philippine (PICCS).

16. OTHER INFORMATION

CURRENT ISSUE DATE: June 21, 2002.

PREVIOUS ISSUE DATE: December 6, 2001.

CHANGES TO MSDS FROM PREVIOUS ISSUE DATE ARE DUE TO THE FOLLOWING:

Amended or modified the following Sections:

- Exposure Guidelines & Skin Protection, Section 8.
- Melting Point, Section 9.

OTHER INFORMATION: **NFPA Classification**
Health: 1
Flammability: 3
Reactivity: 0

Attachment 7. Sample Tag Labeling for Processed Fish Tissue Container

- ◆ Use custom printed labels, or labels supplied with sample jars. If hand writing labels use ballpoint pen or fine-tipped permanent marker (ink that will not run when wet).
- ◆ **MUST BE LEGIBLE!**

Client/Source
Use abbreviated project name in caps.

Site Name
Use pre-assigned "Field Station ID" code in caps. This should match the "Field Station Identification" code on the Lab Analysis & Tracking Plan.

9 Digit Sample #
Place pre-assigned 7 digit work order number from MEL near left side of each label when preparing jars for tissue. The last two digits (xx) represent FFCMP unique sample number 01 - 99.

Common Analysis (note parameter groupings may change)
Use the following abbreviations in caps.

- ◆ PEST-PCB-PBDE-LIPIDS (4 oz. jar – 40g min)
- ◆ Hg (2 oz. jar – 5g min)
- ◆ METALS 14 (2 oz. or 4 oz. jar – 50g min)
- ◆ 3 PCB-3DDT-LIPIDS (4 oz. jar – 40g min)
- ◆ PCDD/F-LIPIDS (4 oz. jar – 40g min)
- ◆ PCB Congener-LIPIDS (4 oz. jar – 40g min)
- ◆ ARCHIVE 1 (4 oz. jar – 10g min, 90g max)
- ◆ ARCHIVE 2 (4 oz jar – 10g min, 90g max)
- ◆ OTHERS – different param groupings may be used. Discuss with MEL prior to processing.

of grams in jar. Note:
4oz jars hold up to 90g.
2oz jars, up to 50g. Do not overfill.

X COMPOSITE for multiple fish combination.
X GRAB for individual fish.

Date/Time
Date sample (fish) was collected.

I - CHEM	90g
CLIENT/SOURCE FFCMP14	<input type="checkbox"/> GRAB <input checked="" type="checkbox"/> COMPOSITE
SITE NAME LOONLMB	DATE/TIME 10/26/14
SAMPLE # 1001015-xx	PRESERVATIVE
ANALYSIS PEST-PCB-PBDE-LIPIDS	COLL. BY

I - CHEM	90g
CLIENT/SOURCE PBDE fish	<input type="checkbox"/> GRAB <input checked="" type="checkbox"/> COMPOSITE
SITE NAME ROCKLSS	DATE/TIME 8/23/08
SAMPLE # 0812012-xx	PRESERVATIVE
ANALYSIS PBDE + Lipids	COLL. BY

Label example for past PBDE project.

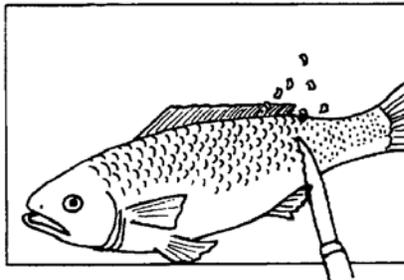
I - CHEM	20g
CLIENT/SOURCE POT - 1 (field ID #)	<input checked="" type="checkbox"/> GRAB <input type="checkbox"/> COMPOSITE
SITE NAME POTSMB	DATE/TIME 10/26/08
SAMPLE # 0812009-xx	PRESERVATIVE
ANALYSIS Hg	COLL. BY

Label example for mercury (Hg) Trends analysis on individual fish.

Attachment 8. Illustration of Basic Fish Filleting Procedure (U.S. EPA, 2000).

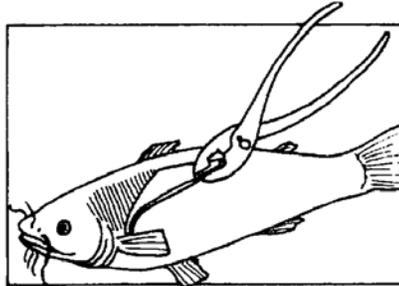
1 Scaled Fish

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:



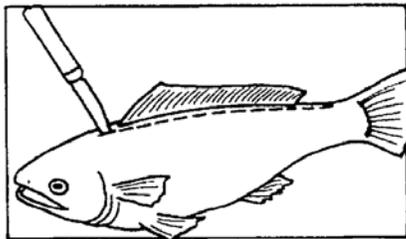
1b Scaleless Fish

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.



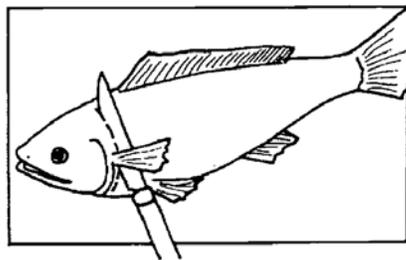
Note: This step applies only for catfish and other scaleless species.

2



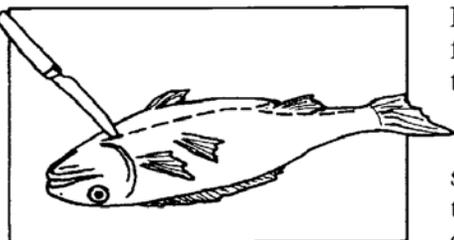
Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3



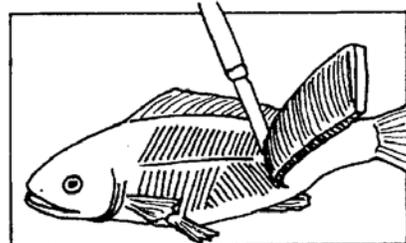
Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

4



Make a shallow cut along the belly from the base of the pectoral fin to the tail. A single cut is made from behind the gill cover to the anus and then a cut is made on both sides of the anal fin. Do not cut into the gut cavity as this may contaminate fillet tissues.

5



Remove the fillet.