NOAA Technical Memorandum NOS OR&R 9

Guidance on Sensory Testing and Monitoring of Seafood for Presence of Petroleum Taint Following an Oil Spill

Seattle, Washington
August 2001

NOAA NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION

National Ocean Service

NOAA Technical Memorandum NOS OR&R 9

Guidance on Sensory Testing and Monitoring of Seafood for Presence of Petroleum Taint Following an Oil Spill

Terriann I. Reilly
National Oceanic and Atmospheric Administration/National Marine Fisheries Service
Seafood Inspection Program

Roberta K. York
Canadian Food Inspection Agency
Laboratories Directorate

Seattle, Washington

Donald L. Evans, Secretary U.S. Department of Commerce	Scott B. Gudes Acting Under Secretary for Oceans and Atmosphere and NOAA Administrator/ Deputy Under Secretary for Oceans and Atmosphere	Margaret A. Davidson Acting Assistant Administrator for Ocean Services and Coastal Zone Management NOAA National Ocean Service

Office of Response and Restoration
National Ocean Service
National Oceanic and Atmospheric Administration
U.S. Department of Commerce
Silver Spring, Maryland

NOTICE

Foreword

After an oil spill occurs, government regulatory authorities and other concerned parties may choose to conduct sensory testing of seafood that may have been exposed. If sensory testing is conducted, it must be carried out in a scientific and legally defensible manner. To date, there are few standard procedures published for this type of sensory testing (Bett et al. 1997). This document was written to provide guidance for conducting sensory testing on seafood suspected of petroleum taint.

Development of these guidelines was supported and partially funded by NOAA/NOS Office of Response and Restoration. The document was written by sensory scientists from the National Oceanic and Atmospheric Administration /National Marine Fisheries Service (NOAA/NMFS) Seafood Inspection Program and from the Canadian Food Inspection Agency (CFIA). In addition to the two principal authors, many other individuals have made significant contributions to this document by communicating practical suggestions gained from their actual work experiences as seafood inspectors and laboratory professionals. The extensive bibliography (Section 9.0) provides supplemental resources for more in-depth information on sensory testing in general and for sensory testing specifically for taint.

These guidelines are the result of collaborative efforts to address the need for standard sensory testing procedures for petroleum taint. By issuing these guidelines, NOAA does not intend to imply that sensory testing should be conducted after every oil spill that potentially involves seafood, or that a closure should be the end-point if taint is detected. The need for sensory testing should be assessed on a case-by-case basis after each oil spill because each spill is unique.

Acknowledgments

Special thanks for the editorial contributions of Ruth Yender and Nancy Peacock, National Ocean Service, NOAA, and for the invaluable information and practical advice from the seafood inspectors of the USDC/NOAA Seafood Inspection Program, and the U.S. Food and Drug Administration.

We acknowledge also the contribution of the technical review committee consisting of: Larry Hufnagle and Dr. Margaret Krahn from the NOAA/NMFS NW Fisheries Science Center, Dr. Alvin Rainosek of the University of Alabama, Mr. James Barnett and Dr. Aladar Bencsath of the U.S. Food and Drug Administration, Dr. Lyle Lockhart from the Canadian Department of Fisheries and Oceans, and Ruth Yender, with the NOAA National Ocean Service.

Contents

Appendices

Appendix 1 Normative References - Existing guidelines, standard practices, and sampling plans for sensory testing.

Appendix 2 Definitions, terminology, and references used in sensory testing for petroleum taint.

Appendix 3 Expert assessor ballot for recording sensory evaluations of seafood: 3-alternative forced-choice (AFC) triangle test.

Appendix 4 Expert assessor ballot for recording quantitative/qualitative sensory evaluations of seafood.

Appendix 5 Trained assessor ballot for recording sensory difference-from-control evaluations of seafood.

Appendix 6 Control graph for plotting and analyzing responses to triangle test for selection of sensory assessors.

Appendix 7 Method for exposing live seafood to petroleum products for purposes of selecting and training sensory assessors.

Appendix 8 Using random code numbers and random-sample presentation methods

Appendix 9 Example of expert assessor training data.

Appendix 10 Example of a Chain of Custody form and instructions.

Appendix 11 Evaluating Expert Assessors Training Results using Fishers Exact Test.

Figures

Tables

1.0
Scope and Use of this Guideline

1.1 Introduction

When an oil spill occurs, local seafood resources may be exposed to petrochemicals that affect their sensory qualities (taste, smell, and appearance). Even when seafood samples from the spill area pass the standard chemical-analytical tests (the levels of polycyclic aromatic hydrocarbons are below the limits permitted as determined by human health risk assessment), flavor or odor still may be affected. Taint in seafood renders it adulterated and unfit for human consumption according to U.S. law (Federal Food, Drug, and Cosmetics Act, US Code 21, Chapter IV, Sec. 402 [342], a.3).

The specific compounds in oil responsible for causing off-flavors and off-odors in seafood have not been determined with certainty. Experience at recent oil spills indicates that, when well-trained sensory panelists conduct sensory testing, there is generally a high degree of correlation between the results of chemical analysis and sensory testing. Because this correlation is not absolute, there is a role for both sensory and chemical analysis in assessing adulteration and safety of seafood. Analytical instrumentation, particularly hand-held electronic noses, continue to advance and may eventually play a significant role at oil spills as tools for rapid assessment. However, at their present state of sophistication, these instruments are chemical detectors only, incapable of making any sensory judgment on odor or flavor. The final judgment about the presence and absence of taint in seafood (or in any food) remains the jurisdiction of human assessors.

Fisheries and water researchers have conducted much valuable research on seafood tainting. However, the pertinent information from the many existing research and guideline documents needed to be pulled together and adapted specifically for testing seafood exposed to petrochemicals. Some of the most extensive research has been conducted in Aberdeen, Scotland at the former Torry Research Station. Individuals from this former institution have provided valuable information included in this guideline. In the literature, some of the most technically useful and inclusive guideline work has been done by Environment Canada, as found in their 1997 *Technical Guidance Document for Pulp and Paper Environment Effects Monitoring* (EEM)

(Environment Canada 1997). Portions of the sensory section of the EEM document served as a framework for writing this guideline.

1.2 Scope and Use

The specific scope of this document is to provide guidance for conducting appropriate sensory tests to objectively assess seafood resources for petrochemical taint following an oil spill. The instruction and background contained here is intended not only for the sensory professionals and assessors, but also for other personnel responsible for managing seafood. This guidance applies to finfish, shellfish/mollusks, and crustaceans.

This document does not provide guidance on how to make the decision to conduct or not conduct sensory testing; it provides guidance on how to conduct the test once the decision has been made to do so.

Seafood issues are unique to each incident. This means that sensory evaluation of seafood resources for petroleum taint must be area- and incident-specific, rather than taking a generic approach to every spill. In addition, the design and implementation of sensory tests for this purpose must generate results that are both scientifically sound and legally defensible. This guideline is meant to help ensure that sensory testing is appropriate to the oil spill context and is conducted with adequate and appropriate quality control. Once the decision to conduct sensory testing is made, it is important that industry and regulatory agencies immediately engage the assistance of an experienced ***sensory professional*** (see **Section 1.3** for definition) in both designing and implementing the sensory-testing procedure.

This guideline provides systematic instructions for sensory testing involving either of two types of assessors:

1) ***Trained assessors****,* or
2) ***Seafood product expert assessors***.

There is a detailed definition of the two assessor types below in **Section 1.3**. Each section of the document is organized according to these two assessor types, as procedures in the section may be different depending on the type of assessor.

This guideline might involve hazardous materials and does not claim to address all of the safety concerns associated with its use. It is the responsibility of the user to

adhere to appropriate safety and health practices. The processing and maintenance of sensory samples should follow good manufacturing practices.

1.3 A few words about terminology

1) The term ***seafood*** generally applies to finfish, shellfish/mollusks, and crustaceans. When sections of this guideline refer only to one of these classes, it will be specified.

2) ***Tainted seafood*** is herein defined as ***"seafood that contains abnormal odor or flavor"*** based on the sensory analysis vocabulary of ISO 5492 (see **Appendix 1**). The contextual meaning of ***taint*** is an odor or flavor introduced into the seafood from external sources.

   This ***excludes*** any natural byproducts of deterioration due to

- aging during storage, i.e., decomposition of fats, proteins, or other components; or
- microbial contamination normally found in fish.

3) In the field of Sensory Science, many terms are interchangeably used to refer to certain ***personnel*** involved in a sensory test. For purposes of this document, the terms will be defined as follows:

- ***Sensory professional*** will be defined as an individual who has received a combination of University-based sensory science instruction and practical experience that provides them with the ability to design and execute various types of sensory studies.
- ***Assessor*** is defined as any person taking part in a sensory test, usually an individual who evaluates a sample for odor, taste, appearance, etc.
- ***Panel*** is defined as any group of assessors chosen to participate in a sensory test. Following, different types of assessors are defined in order of experience and level of training:
    a) A ***consumer*** is defined as an untrained, naive assessor.
    b) A ***trained assessor or trained panel*** is defined as a group of assessors selected and trained to perform a specific task (this may be for a particular product or for a particular attribute). For purposes of

this guideline, they will hereafter be referred to as **"trained assessors".**

c) An ***expert assessor*** is defined as an assessor with a high degree of sensory ability and experience with sensory methodology and who is able to make consistent and repeatable sensory assessments of various products.

d) A ***specialized expert assessor*** is defined as an individual who has additional experience as a specialist in a certain product and/or process and who and is able to evaluate the effects of variations related to raw materials, recipes, processing, storage, aging, etc. In many countries, such as the US and Canada, seafood inspectors (employed by either NOAA/NMFS or USFDA or the Canadian Food Inspection Agency (CFIA)), perform sensory testing of a wide variety of seafood species and processes on a daily basis as part of their job. In addition, most inspectors have received some education in sensory science methodology, and have been selected, trained and validated as being highly accurate and repeatable sensory assessors. These individuals meet the requirements of *specialized expert assessors* of seafood products. For purposes of this guideline, they will hereafter be referred to as **"expert assessors".**

This document provides guidance on sensory testing using either a panel of **"trained assessors"** (b) *or* "**expert assessors"** (d). Further details of the differences in selection, training, testing methodology and numbers of assessors needed are addressed in each section of this guideline.

Additionally, see **Appendix 2** for a detailed list of definitions of sensory attributes used in this document.

2.0
Collection and Preservation of Seafood Samples for Sensory Evaluation

2.1 Introduction

This section provides technical guidance on the collection and handling of seafood samples for testing the sensory effects of petrochemicals on the resident finfish and shellfish populations. Sensory professionals should collaborate with other involved individuals such as fisheries biologists, statisticians, spill site managers and coordinators, and appropriate regulatory authorities, on planning the number and size of sampling sites, the number of organisms from each site to be tested, sample collection responsibility, and chain of custody procedures.

See **Section 2.7** for more details or **Appendix 1** of this document for examples of sampling plans.

2.2 Types of samples to be collected

Several types of samples are referred to in this guideline:

- ***Samples*** generally refer to indigenous species collected from the spill area to be tested for the presence of taint, i.e., the “unknowns” for which the qualities of odor and flavor will be established. These could also include living seafood samples that are held in captivity in the spill area (generally in cages) to monitor changes in taint as the spill is cleaned up and the oil begins to weather, or in commercial aquaculture facilities.
- ***Control samples*** include any seafood samples taken from unaffected areas adjacent to the spill area to provide the "background fish flavor/odor against which taint in the “unknown” samples is assessed. Control samples are collected before or at the same time as "affected" samples from the spill area and should be handled in exactly the same manner. They provide an internal control in the test design, and the data from these samples can be used to measure the degree to which samples from the spill area differ from background samples. Control samples of each species to be tested *should* be included in the test design when using expert assessors, but *must* be included when using trained assessors. When control samples are not available under any circumstances,

sensory assessments should be conducted only by expert assessors trained in evaluating the species in question who, therefore, have an "internal control" from their training and on-the-job experience.

- ***Concurrent samples for chemical analysis*.** In addition to sensory testing, it is sometimes decided to conduct chemical testing of seafood samples from the affected site. If the decision is made to conduct chemistry, the final sample size should include enough samples from the affected and control areas to allow one-half of each organism to be retained for chemical analyses, and for possible correlation with sensory analyses. The same rapid collection and preservation methods should be used in handling these samples, along with the same thorough documentation and chain of custody, as for sensory samples. In addition, because organisms are usually not tainted uniformly in any environment, it is important to code samples so that chemistry and sensory data can be compared **for the same organism**. Chemical analyses can sometimes be delayed until results of sensory testing are available.
- ***Reference samples*** are used in sensory training and testing as an illustration for the definition of a sensory attribute or condition (such as intensity). These include any product that illustrates a sensory attribute or intensity and, in this work, may include seafood, petroleum products, other food or non-food items, etc. (see **Appendix 2**). Reference samples are chosen so that they demonstrate a chosen attribute and can be generated as often as needed and in the same “repeatable” condition. Data are not collected from reference samples, nor are they included in the test design for statistical analysis.

2.3 Timing of sampling during oil spill and cleanup

The time between exposure of an organism to spilled oil and the onset of tainting varies, as does the intensity and persistence of the taint (Motohiro 1983; Law and Hellou 1999; Whittle et al. 1999). The degree of taint depends on several factors: type of petroleum product, habitat, water temperature, weather conditions, etc. (see Motohiro 1983, for a review). Reported times required for onset of taint range from less than 1 hour to 48 hours; thus samples for sensory testing should not be taken before a minimum 48 hour exposure period.

Control samples from areas adjacent to the spill *should* be taken as quickly as possible (no waiting period) and *must* be taken before the spill spreads. For obvious reasons, control samples and affected samples must be taken from an area as close as possible to the affected area to reduce background variability in natural sample odors and flavors. **Figure 1** schematizes the necessary steps in collecting, handling, and shipping seafood samples from the spill site to the evaluation laboratory.

2.4 Handling samples during and after collection

It is of primary importance that all samples be collected to prevent any post-sampling exposure to contaminants or other conditions that could affect the results or credibility of the sensory assessments. For example:

- Exposure to fuel or other petroleum products aboard the sampling boat
- Contact with packaging materials made with petroleum products
- Exposure to inappropriate holding temperatures or conditions that could induce the production of off-flavors or off-odors within the product

It is essential, from the start, to ensure that all necessary steps are followed to demonstrate "chain of custody" (see **Section 2.6.2**). To be legally valid, it must be evident that the data were generated from samples taken from the specified areas and could not have been contaminated at any other point in time after sampling.

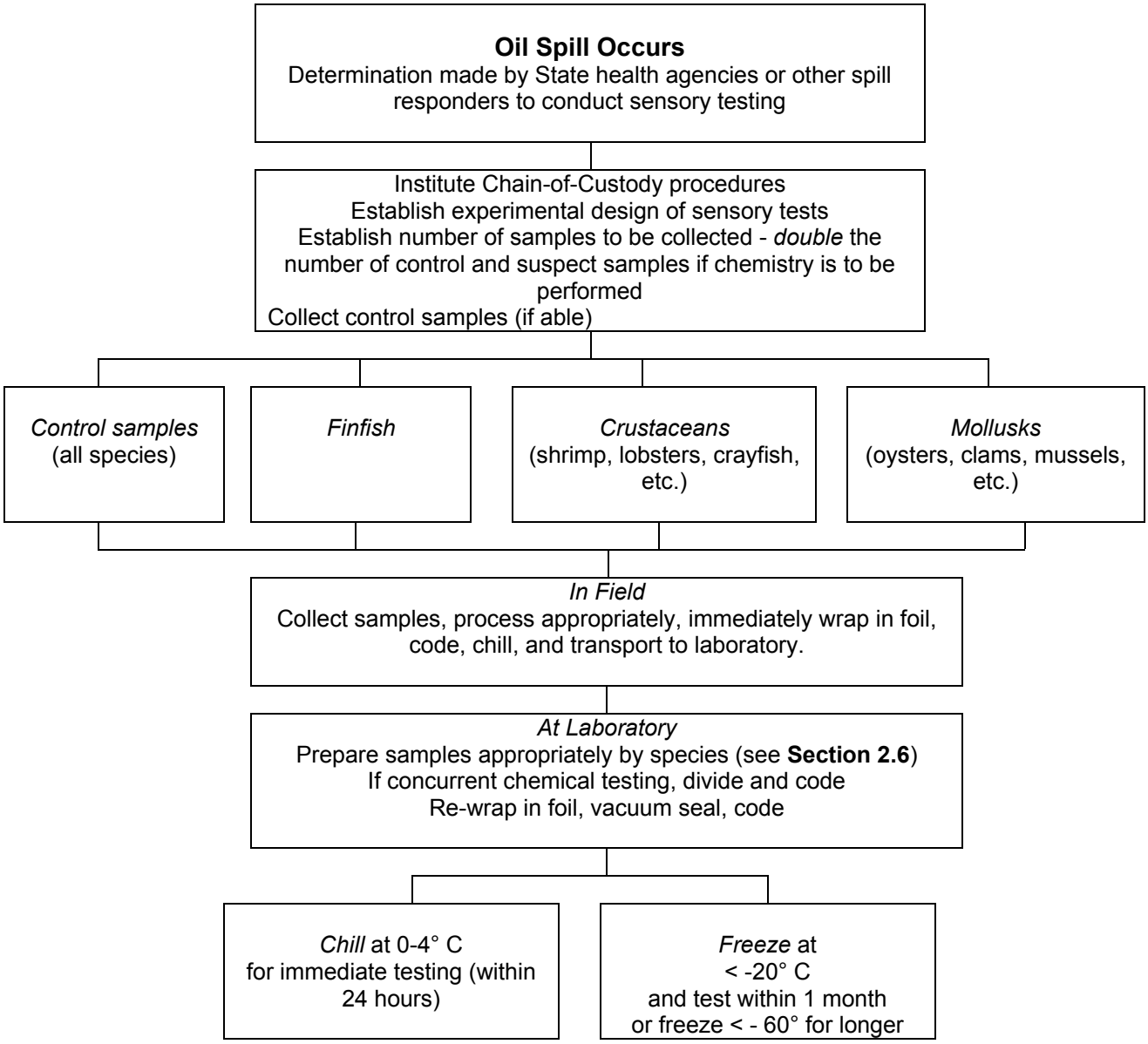


Figure 1. Summary of sample collection, handling, and shipping for sensory evaluation.

2.5 Ready-to-use sampling kit

As part of emergency preparedness, a sampling kit can be pre-assembled and ready for field use when an oil spill occurs. We suggest the contents of such a sampling kit as shown below.

Quantity for sample size *n*=21 organisms	Items
1 roll	Heavy-duty aluminum foil - institutional rolls.
25	Vacuum-packaging bags of a size appropriate to the species being sampled. Pre-code if possible.
1	Vacuum sealer (consumer model, if institutional model is not available).
50	Zip-lock bags of suitable size, with straws for evacuating air. Pre-code if possible.
6	Cutting boards (use separate boards for control and exposed samples; label clearly).
6	Knives (use separately for control and exposed samples; label clearly).
4	Scissors (use separately for control and exposed samples; label clearly).
4	Permanent marking pens
25	Adhesive labels
2	Coolers (1 each for control and exposed samples; label clearly).
6 sheets	Styrofoam or packing material
1 roll	Newsprint (unprinted) for packing material
2	Shipping cartons (1 each for control and exposed samples).
weight of samples	Dry ice or ice packs
2 rolls	Packing tape and/or masking tape

Figure 2. Sampling kit for collection of sensory samples.

2.6 Chain of custody and ensuring validity and integrity of sensory samples

2.6.1 Collecting seafood samples

The EEM document (Environment Canada 1997) cites seven requirements for sample collection of indigenous species for exposed and control samples that can be adapted to sampling during oil spills.

1) Both ***exposed*** and ***control*** samples must be collected from the same water body and similar habitat type. Though the EEM document applies to collecting from smaller freshwater areas, the same principle holds true in marine sampling. This reduces any variability in flavor caused by seafood samples originating from dissimilar habitats.

2) The same collection methods must be used in obtaining both exposed and control samples, and organisms must be removed from the water as quickly as possible after capture.
3) Organisms collected in the control area must be physically isolated from the exposed population to prevent any mixing of the two groups.
4) Exposure times for organisms collected in the exposed area should be at least 48 hours; to ensure that uptake of oil has reached its maximum.
5) Standard measurements, such as weight, length, sex, and age, must be recorded for each organism collected for sensory testing. If possible, steps may be taken to preserve the otolith for age determination.
6) Organisms taken from each exposed and control area should approximate the same size, age, and sex ratio. If *trained* assessors are used, the weight range among organisms should not exceed a factor of 1.2 to minimize potential differences in texture, color, and flavor. This is not an issue for expert assessors, providing sample sizes are large enough to allow multiple assessors to evaluate them.
7) Only organisms that are alive at the time of collection should be used for testing to ensure that they have been exposed to taint conditions. This also ensures that there are no flavor effects from decomposition present, as fish can deteriorate while still in the water if they die in the net (EEM and ASTM documents, see **Appendix 1**). Any quality (freshness) evaluations must be performed **only** by trained inspectors or expert assessors.

2.6.2 Ensuring chain of custody (continuity of evidence)

During sample collection and preparation for transport, the handlers must be able to account for all aspects of handling in case they are called to testify in legal proceedings (see part 2 of **Appendix 10** for a definition and detailed list of procedures). Complete documentation must be demonstrated through detailed note-keeping, including the time of all significant events, where event occurred, what happened, who is present, etc. This also includes being able to swear under oath as to the location of the samples from the moment they are collected until the samples are delivered to a specific individual at the sensory testing laboratory. From that point on, chain of custody becomes the responsibility of the sensory testing laboratory (although, in some cases,

the individual who supervised the sample collection may be the same person responsible for sample storage and preparation at the laboratory).

- Ensure security of the sample at all times. If the samples are left untended for any period of time, (e.g., while being held in chilled or frozen storage), they should be locked in an appropriate container and a seal placed around the opening with a signature to show that the container was not disturbed (opened). An example of this would be holding the samples in a cooler, with the lid taped around to completely seal it into place and with signatures of the responsible person on the tape or seal.
- Record the identity of every person who has custody of the samples at any point in the sequence, and include an NRDA-style chain-of-custody form. This number should be kept to a minimum with the ideal number being one individual (or, at the most, two), as each individual may possibly be required to testify as to the sample security.
- Each party involved should record the time and other pertinent details, such as when samples are transferred from individuals and locations.
- Record the location and environmental conditions under which samples are held throughout the entire history, e.g., temperatures for chilled or frozen samples.
- An example of a chain of custody form and related instructions are included in **Appendix 10**.

2.6.3 Secure shipping of frozen samples.

If sensory testing cannot be performed within a 24-hour period, samples should be frozen according to procedures specified in **Section 2.8**. Instructions are given here for the secure transport of frozen samples to the sensory laboratory. It is imperative that chain of custody be maintained during this process.

- Samples should not, at any point, reach a temperature above -20° C during shipping or storage.
- Initially, all seafood samples should be wrapped in aluminum foil to ensure no contact with petroleum-based packaging materials.
- The foil-wrapped samples should then be vacuum-sealed and then over-wrap again with heavy-duty aluminum foil. Make sure each sample is clearly labeled.
- Place clear, odor-free shipping carton lined with thick Styrofoam and packing material in the freezer to chill before adding the samples.

- Dry ice or ice packs will be added to the shipping carton with no less than a 2:1 ratio with samples. Alternate layers of sample with layers of ice packs or dry ice. The box should be as tightly packed as possible to exclude as much air as possible. Pack with unprinted newspaper (i.e. clean, never exposed to ink) or with Styrofoam chips to fill the air voids. As many air voids as possible should be excluded to maximize the frozen stability of the samples.
- Seal package tightly, keeping in mind the requirements for chain of custody (i.e., securing and signing the closures so that evidence of any tampering will be clearly apparent). If possible, photograph the sample containers prepared for shipping. Ship immediately via secure transport. Package should not be en route more than 24 hours.
- Upon receipt of the sample container, examine it for security of contents, i.e., any evidence of tampering, and document condition of container. If possible, photograph the sample containers on arrival. Then, open the container, remove samples from extra packing material, but retain vacuum packaging and immediately place in secure freezer at -20° C or lower. Examine samples for any signs of thawing and document accordingly.

2.7 Statistical sampling requirements

The numbers of each species that should be collected from any given area for purposes of sensory testing depends on: 1) the population size of the species and the size of the affected area (calculated according to the best-available information) or, 2) on the minimum number required that will give a sufficient quantity of sample for testing. The assumption is always made that there will be some variability in uptake of taint within a species when exposed to the same tainting conditions.

There are two considerations in establishing the number of samples required for testing:

2.7.1 Basic types of statistical sampling plans

- ***Attribute*** Each sample unit is classified according to conformity.
- ***Variable*** Characteristics are evaluated on a numerical scale.

For an oil spill situation, attribute-sampling plans are recommended. Thus, if a sample is tainted, it is non-conforming.

For attribute sampling guidelines, refer to the 1994 U.S. Code of Federal Regulations, Title 50; the 1993 ANSI/ASQC attribute sampling procedures; the UN/WHO Codex Alimentarius Commission sampling procedures; and the ISO attribute sampling procedures. See **Appendix 1** for complete citations.

2.7.2 Required sample sizes and presentation for sensory testing

- At least 6 samples per species *from each area* are recommended for testing with either type of assessor. As a general rule, the sample size collected should comply with the minimum requirements in the statistical sampling tables of ISO, Codex Alimentarius, CFIA, and NOAA (see **Appendix 1**). If samples are to be collected concurrently for both sensory and chemical analyses, the sample size should be *doubled* to ensure sufficient samples.
- For testing by ***expert assessors:*** A sample will consist of an individual organism when testing finfish and lobsters, or multiple organisms when testing shellfish. Depending on the size of the shellfish, 3 to 6 organisms are recommended for a sample.
- When using ***trained assessors:*** Samples can be presented in one of two ways.
  - ***20-g blended, individual fish samples*** (BIFs) from multiple pooled organisms, *or*
  - ***20-g dorsal-muscle single-organism samples*** (see Environment Canada 1997). Given the expected recovery of fish or shellfish flesh (see table below), you will need to compute the total weight of the final sample and the estimated number of samples needed.

Type of seafood	Expected % recovery of edible flesh
Finfish	38–40
Flatfish	30–33
Lobsters	14–18
Shrimp	28–30
Clams	16–20
Oysters	25–30
Scallops	20–25
Mussels	15–20

2.8 Species-specific collection procedures

Samples must be obtained for each commercial species normally harvested from the area that is suspect. Generally applicable instructions for all samples should be: 1) handle in a manner that ensures that no interfering odors/flavors of decomposition are allowed to develop that could interfere with the evaluation of flavors from contaminants, 2) do not expose to products of petrochemical origin that might interfere with the integrity of the sample under chain of custody. The goal of these methods is to immediately stabilize the harvested seafood samples. The important factors are cleaning, protecting from exposure to air, and chilling. Following are the general requirements for each type of seafood.

2.8.1 Finfish

Samples should be taken from the water source using clean gear, not previously exposed to petroleum from any source.

- Seafood samples are rinsed in clear, potable, odor/flavor-free water to remove any residual surface oil (if necessary, suitable bottled water can be brought to the sampling site).
- Samples are to be prepared in the same manner as for commercial sale. Clean to remove viscera, gills, and kidney if this is done in commercial practice. Under no circumstances should the viscera come into contact with the flesh that will be used for sensory evaluations. The head should be left on but the gills removed carefully to preserve the otolith if it will be examined for age determination.
- Carcasses must be rinsed in clean water, such as distilled water or other clean potable water source, and immediately placed on non-chlorinated ice with the body cavity facing downward to allow for drainage. Use enough ice so that the sample does not come in contact with drainage or melt water.
- After draining, samples should be tightly wrapped in double layers of heavy-duty aluminum foil and then individually placed in double zip-lock bags, evacuating as much of the air as possible by pushing it out or by sucking it out through a straw. Ensure that no plastic touches the sample. Ideally, the samples should be vacuum-sealed if they are to be frozen (several home models are available).

- Samples must be clearly labeled with all pertinent information regarding sampling sites, dates, etc. (see **Section 2.6.2**). All labeling should be firmly and securely affixed to the plastic outer-wrap of the samples.
- Either chill samples to 4º C and test within 24 hours, or freeze samples immediately and store at a temperature of at least -20º F (preferably -30º F).
- Frozen samples must remain frozen at all times during shipment from the field to the sensory-evaluation laboratory, or well iced if the testing will be done within 24 hours.

Samples should be tested as soon as possible and not kept frozen for more than 1 month before testing. (However, with proper handling and < - 60º F storage, samples can be saved for longer periods and used successfully to demonstrate the presence of taint.). To maximize ease of data presentation, we recommend shorter rather than longer storage times. If samples are to be in frozen storage and are not vacuum-sealed in the field, they should be vacuum-sealed upon arrival at the sensory-evaluation laboratory as described above in this section.

2.8.2 Crustaceans and molluscan shellfish

Ideally, a suitable sensory laboratory would be available near the spill site so that crustaceans and mollusks can be evaluated alive, as they would be just before cooking by the consumer. If this is not the case, samples are to be shipped alive or frozen to the evaluation laboratory as described below**.**

The goal of these methods is to immediately stabilize harvested shellfish to prevent any changes due to deterioration that could interfere with the evaluation of petroleum-related flavors and odors. The important factors are cleaning, protecting from exposure to air, and chilling with ice (if samples are to be tested alive) or by rapid freezing if live testing is not possible.

- Samples must be rinsed in clean, potable, odor- and flavor-free water (e.g., distilled or filtered) to remove any residual surface oil and immediately placed on non-chlorinated ice. Sufficient ice should be used to keep samples from coming in contact with melt water.
- Samples should be coded in the field with unique 3-digit random numbers (see **Appendix 8**) and labeled with all the pertinent information.

- Samples that can be evaluated alive should be tightly wrapped in double layers of heavy-duty aluminum foil and placed on ice in clean, odor-free shipping containers.
- Samples that must be shipped frozen for evaluation should be wrapped individually in double layers of heavy-duty aluminum foil, and frozen at -20º C, or lower for about 30 minutes to immobilize the sample (i.e., lobsters, crab). They are then placed in double zip-lock bags with as much of the air evacuated as possible by pushing or sucking it out through a straw, ensuring that no plastic touches the sample. Ideally, samples should be vacuum-sealed before freezing.
- Samples must be clearly labeled with all pertinent information required coding each sample for later analysis at the sensory-evaluation laboratory. All labeling should be firmly and securely affixed to the plastic outer-wrap of the samples.
- Again, all appropriate measures must be taken to ensure chain of custody throughout the handling and transportation steps.

3.0
Selecting Appropriate Sensory Test Methods and Assessors

3.1 Introduction

Because of the legal and regulatory implications of oil spills, it is strongly suggested that the sensory evaluation of seafood be designed and conducted under the direct supervision of a trained professional, in this case a ***sensory scientist*.** Most importantly, consideration must be give to the amount of information that interested parties will require and the types of assessors that are available. The level of necessary assessor training and the extent of information that can be collected will vary according to the selected test method.

3.2 Subjective vs. objective sensory testing

Sensory evaluation in general can be either **subjective** (consumer) or **objective** (analytical). Some key differences in subjective vs. objective sensory test methods include: the type of assessor used (untrained vs. trained), types of information collected (personal feelings vs. product attributes and intensity), and numbers of assessors needed (many vs. few). When evaluating any food for the presence of taint, in this case seafood, the evaluations must be ***objective***.

3.2.1 Subjective testing

Subjective testing measures responses as ***feelings and preferences*** about samples. ***Untrained assessors*** on this type of consumer panel provide purely subjective responses—based on personal bias—which are not appropriate for taint analysis. Subjective sensory evaluation measures an assessor's feelings toward a sample; it does not measure the sensory attributes of the sample. Thus, subjective testing is clearly NOT appropriate for assessments of taint upon which decisions to close/reopen fisheries may be based. These kinds of decisions call for objective, analytical sensory evaluation to confirm and quantify the presence of taint (York 1995).

3.2.2 Objective testing

Objective sensory testing measures the ***intrinsic sensory attributes*** of a sample through the analytic sensory perceptions of trained human assessors (Jellinek 1985; Meilgaard et al. 1999). Several objective test methods would be suitable for assessing the presence of seafood taint.

3.3 Selecting a sensory testing approach – general considerations

3.3.1 What answers are required?

From the start, interested parties, working with a sensory professional, must decide what they want to know – what questions do they want answered so that the appropriate test method can be selected. Each test method requires different:

- numbers of assessors
- assessor training periods
- data collection methods
- repetitions of the test
- sample numbers

Difference testing will result in basic information that would answer the question "is there a difference between the suspect and control samples?" but does not provide information on the nature or the degree of the difference. More sophisticated testing, such as descriptive analysis may answer many questions such as; "Is there a difference What is the difference? How big is the difference? Is the difference due to petroleum taint? How intense is the taint? What is the description of the sensory characteristics of the taint?" In general, the tests that give you more information about the samples require greater assessor training periods, but less numbers of assessors, test repetitions, and sample quantity. These methods will be further explained in this section.

3.3.2 Assessor type

Objective sensory measurements are obtained from the following three types of trained assessors:

1) Assessors ***screened and selected*** **for** sensory tasks (usually 25),

2) Assessors ***selected and highly trained*** to participate on a ***panel*** for ***specific sensory tasks*** (usually 10 to 15),
3) ***Expert* assessors**, e.g., product specialists, seafood inspectors (usually 1 to 5) (York & Sereda 1995).

As the list of assessor types progresses above, the level of training and experience increases which then increases the amount of information that that may be obtained from the test. To conduct any ***objective*** sensory analyses of seafood (or any product), assessors must be

- ***selected*** according to their abilities to perform the sensory tasks at hand,
- ***trained*** in the application of the required test methods, and
- ***monitored (validated)*** for their ongoing abilities to effectively perform the sensory tasks.

Assessors are chosen to work within specific test protocols and must be trained to perform within those criteria (see **Table 1**). Assessors must be considered the analytical tool for the assessment for the presence of taint.

3.3.3 Numbers of assessors required

In general, the higher the level of training, the less statistical variability in the sensory data and the fewer the number of assessors required. (Note: This is why very large panels, of 100 or more assessors, are required for subjective (consumer) testing. Because their responses are personally biased, there is wide variability within and among the resulting data sets.)

In many cases, the type and number of assessors available (professionals, expert/trained, new trainees) limit the feasibility of testing approach that can be implemented.

3.3.4 Assessor availability

In reality, the limiting factor in choosing a particular sensory test method following an oil spill is often the type of assessors available, given the urgency and often remote locations of spill scenarios. With this in mind, two types of assessors that are recommended are:

Expert assessors can specify the presence and intensity of petroleum contamination, as well as the presence of flavor changes that are not due to taint (i.e. quality changes due to autolytic and/or microbial decomposition).

Trained assessors can measure the degree to which samples differ from standard, uncontaminated samples for the presence of taint. Because this group is not trained in assessing general seafood quality, we cannot assume that they will differentiate petroleum taint from other quality changes, caused perhaps by deterioration, that might be present in seafood with low or borderline levels of taint. For this reason, it is even more essential when using trained assessors that control samples and taint samples be handled and processed quickly to prevent extraneous flavor changes.

3.4 Test selection

3.4.1 Discriminative testing

Discriminative testing is also referred to as difference testing. Several different sensory test methods will allow one to determine differences. The 2 types of test below are appropriate for taint assessment.

3.4.1.1 Difference testing using the Triangle Test

In early studies to measure the effects of petrochemical exposure on fish flavor, one of the sensory-evaluation methods used is difference testing using the *triangle test*. Here, an assessor is presented with three coded samples—two of which are the same—and is asked to identify the "odd" sample. This is known as the ***3-alternative forced-choice triangle test***.

There are ***problems***, however, in using the triangle test to establish the presence of petrochemical taint.

- The procedure is used to determine *any* perceptible sensory difference between samples of two products; thus, all differences—not just the presence of petrochemical taint—will be used by the assessor in evaluating the samples (e.g., color, texture and other flavor differences which may not be taint).
- Actual differences among samples may be detected in a single sensory attribute or in several attributes.

- Although applicable when the nature of the difference is unknown, this test does not determine the *magnitude* or *direction* of difference; also, assessors must be trained to identify the attribute responsible for the difference.
- The test is applicable *only* if the products are homogeneous, and does not account for natural variations in the product, such as those found when working with seafood.
- Because the test identifies only a difference, and not the magnitude of the difference, the data *cannot be compared over time*, such as when the effects of the petrochemical exposure begin to abate.
- Triangle tests require a relatively larger quantity of sample than the recommended test.

3.4.1.2 Difference-from-control (DFC) testing

The DFC test is recommended for use with *trained assessors, and sometimes expert assessors* in assessing petroleum taint in seafood flesh (ASTM E 1810-96; **Appendix 1**). This test is often used in quality-control situations to measure a difference and estimate the size of any difference found, taking into account the natural variability of the product. The *advantages* of this method include:

- provides an estimate of the presence and degree of difference compared to a clear control;
- defines more easily the nature of the difference;
- assessor is less influenced by other factors in the sample, e.g., natural flavor of the seafood and intra-species flavor differences among samples from different areas;
- data collected can be analyzed through standard statistical tests;
- test recognizes that seafood, being natural foods, have some variation in flavor and this natural variability is considered in the experimental design and the statistical analysis;
- comparisons between the triangle and DFC tests show a high frequency of *false* statistically significant results (Type I error) in the triangle test (Aust et al. 1985).

3.4.2 Descriptive Analysis Testing

Descriptive analysis is a sensory method by which the attributes of a food or product are identified and quantified, using human subjects who have been specifically trained for this purpose (Manual on Descriptive Analysis Testing for Sensory Evaluation, ASTM Manual Series: MNL 13, 1992). Most of these methods require the use of highly trained assessors and evaluate many sensory attributes within each sample (York and Sereda, 1995). For taint assessment, panelists can be trained to provide both qualitative and quantitative information on the presence of petroleum taint only. In petroleum spill or environmental effects monitoring situations intensity (quantitative) data may provide valuable information to the interested parties if the problem persists for long periods of time (Whittle et al. 1997).

3.4.3 Testing with Product Experts and official inspections

The International Organization for Standardization (ISO), Technical Committee 34 on sensory analysis, sub committee 12 has accepted the role of the expert assessor in product evaluations (see ISO/DP 8586 Sensory Analysis - Assessors, Part 2 - General Guidance for the Selection, Training, and Monitoring of Experts). Experts contrast with other types of assessors in the level of training that they must have in both product specialization and sensory analysis methods, and also in their responsibility for samples, testing conditions, data analysis, and reporting of results (York and Sereda, 1995). This awareness of the need for expert assessors' seafood evaluation for international trade has been demonstrated by Codex Alimentarius in a Code of Practice for the Sensory Evaluation of Fish and Fish Products (see **Appendix 1**).

Government agencies routinely use seafood product experts to inspect seafood. These inspectors undergo years of training and direct product experience and may easily be further trained to detect the presence of taint, such as from an oil spill. These assessors use either a pass/fail or a grading system, but, in either case, seafood that is tainted or unwholesome is considered not fit for consumption or trade and will not pass an inspection.

3.5 Selecting an assessor type

3.5.1 Two types of assessor panels appropriate for seafood taint assessment

- ***Panel of 3 to 5 expert assessors*** (fish inspectors, usually employed by a regulatory agency) whose job it is to assess fish for its suitability for sale for human consumption. These assessors must be selected for and have additional training to detect petroleum taint in seafood.
- ***Panel of 10 to 15 selected and trained* assessors** convened specifically for the task of assessing taint from a particular oil spill.

The capabilities of different types of assessors and the advantages and disadvantages for taint assessment in particular are compared in the following table [see also ISO 8586-2:1994(E); see **Appendix 1**].

Table 1. Characteristics of various types of sensory assessors in general.

Type of Test	Type and definition of assessor	Characteristics of assessors and numbers needed	Possible advantages of using such assessors
Consumer Tests	Untrained, naive assessors chosen to represent market segment.	Untrained - Respondents relate feelings, wants and needs of products. Need at least 100 respondents.	Not appropriate for taint assessment.
Discriminative Tests	Selected and trained assessors who have been screened and selected for. Chosen for his/her short-term ability to perform a sensory test.	Assessors work under supervision of a sensory professional and perform short-term tasks such as difference testing and scaling. Need approximately 25 respondents.	More readily available, esp. in remote areas. Only spill-specific training is required.
Descriptive Tests	Assessors who have been screened and selected for and extensively trained for long term tasks.	Experienced assessors who also work under the supervision of a sensory professional. Consistency of judgment, both within and among testing sessions. Can provide detailed quantitative and qualitative information Need 10-15 respondents.	Fewer assessors required to maintain a given degree of reliability in the results. Long-term sensory memory and accumulated experiences allow recognition of particular attributes, such as taints.
Tests using Product Experts as assessors	Assessors with high degree of sensory sensitivity and well experienced with sensory methodology. Specialized experience with the product and/or the process and/or marketing and can make consistent and repeatable sensory assessments. One who can evaluate or predict effects of variations relating to raw material, recipes, processing, storage, aging, etc., of various products.	Long-term sensory memory. Can operate independently. Extensive experience in the relevant specialist field. Highly developed ability to recognize and evaluate sensory properties. Mental retention of reference standard. Recognition of key attributes. Can apply deductive skills to problem solving and can describe and communicate conclusions or take appropriate action. For taint assessment, 3 to 5 respondents are recommended.	Evidence from an expert panel is more persuasive, for example, in a court of law. A product expert assessor takes full responsibility for all judgements, comments and estimates, including tasks undertaken by a panel leader. Gives advice on sensory aspects of contractual or legal matters.

3.5.2 Recommended assessor types

3.5.2.1 Expert assessors

Expert assessors are most preferable because they are trained, experienced, and validated (sometimes certified) in assessing seafood products. Trained assessors will require years of more training to achieve the same goals. Expert assessors must be further selected and trained specifically for petroleum tainted seafood (see **Section 1.3**).

- If sensory results indicate that commercial seafood resources are tainted, it may have economic implications to individuals and industries for extended periods. Considering both the potentials for damage claims by affected parties and the speed with which the spill response must be handled, *we recommend that, when possible, expert assessors be chosen.* These inspectors are already familiar, through their training and experience, with the sensory attributes of taint. ***Seafood inspectors*** are employed by government agencies and assigned to specific offices and laboratories in different areas of the country. By prior agreement with their employing agencies, arrangements can be made for temporary assignments of these seafood inspectors to oil spill-related sensory analysis in an emergency situation. This means they can travel to the spill area or sensory laboratory on short notice and immediately begin assessing spill-related taint of seafood stocks. The presence of a ***defect*** can be quantified by these inspectors, as in seafood inspections carried out under U.S. federal law where ***defect levels*** are established.
- Although seafood inspectors are experienced in assessing all aspects of quality deterioration in seafood, *we recommend that the sensory testing methodology be designed to generate additional data other than in routine inspections* (see **Section 8.3**). The first step in this testing should be to re-familiarize (recalibrate) the inspectors with the sensory attributes and intensity of petroleum contamination.
- ***Canada's approach*** to sensory testing differs in that descriptions of *defects* under the categories *tainted, decomposed and unwholesome* are given by law. Also, a *defect* is defined as *perceptible at the level of "distinct and persistent,"* in other words, a flavor or odor that may be present at a very low level, but that does not disappear when left to stand for 1 minute.

3.5.2.2 Trained and monitored assessors

Trained and monitored assessors are the other choice for objective sensory evaluation. Ten to 15 individuals are selected according to their consistent ability to perceive taint at normal levels, and then trained for these assessments. Using trained assessors requires the constant application of a sensory test with samples *relative to a known, clear, control standard*, for presence of a defined difference, e.g., the difference-from-control (DFC) test. In addition, when using trained assessors, every aspect of the training and testing must be conducted in the light of potential regulatory and legal impacts and the reliability of the evaluation results. Time and resources must be allocated to monitor and validate the performance of trained assessors to ensure that the data produced will be allowable in court, if required.

3.6 Criteria for selecting *expert* and/or *trained* assessors

To be selected either as an expert assessor or for a trained assessor panel, candidates must meet the following qualifications:

1) Basic sensory acuity and the ability to describe perceptions analytically (i.e., not influenced by personal bias). Allergies to seafood or to some food additives should eliminate a candidate assessor.

2) Potential to develop *analytic capability* through familiarization with test procedures, to increase ability to recognize and identify sensory attributes in complex food systems, and to refine sensitivity and memory so that the assessor can provide precise, consistent, and standardized sensory measurements that are reproducible.

3) Capable of being *monitored* (validated) through frequent, periodic evaluations of the performance and consistency of his/her sensory-analysis decisions.

3.6.1 Time required to assemble a panel of assessors

If expert assessors have been previously trained for petroleum taint, they will require 4 to 6 hours on the day prior to sensory testing to re-calibrate. For trained assessors, the best scenario would be to have a previously trained and recently validated panel available. Assuming that a specific petroleum taint panel of trained assessors does not already exist, approximately 3 weeks will be needed prior to sensory testing to recruit, select, train, and validate a panel of assessors. See **Figure 3** for a decision tree for selecting sensory testing methodology.

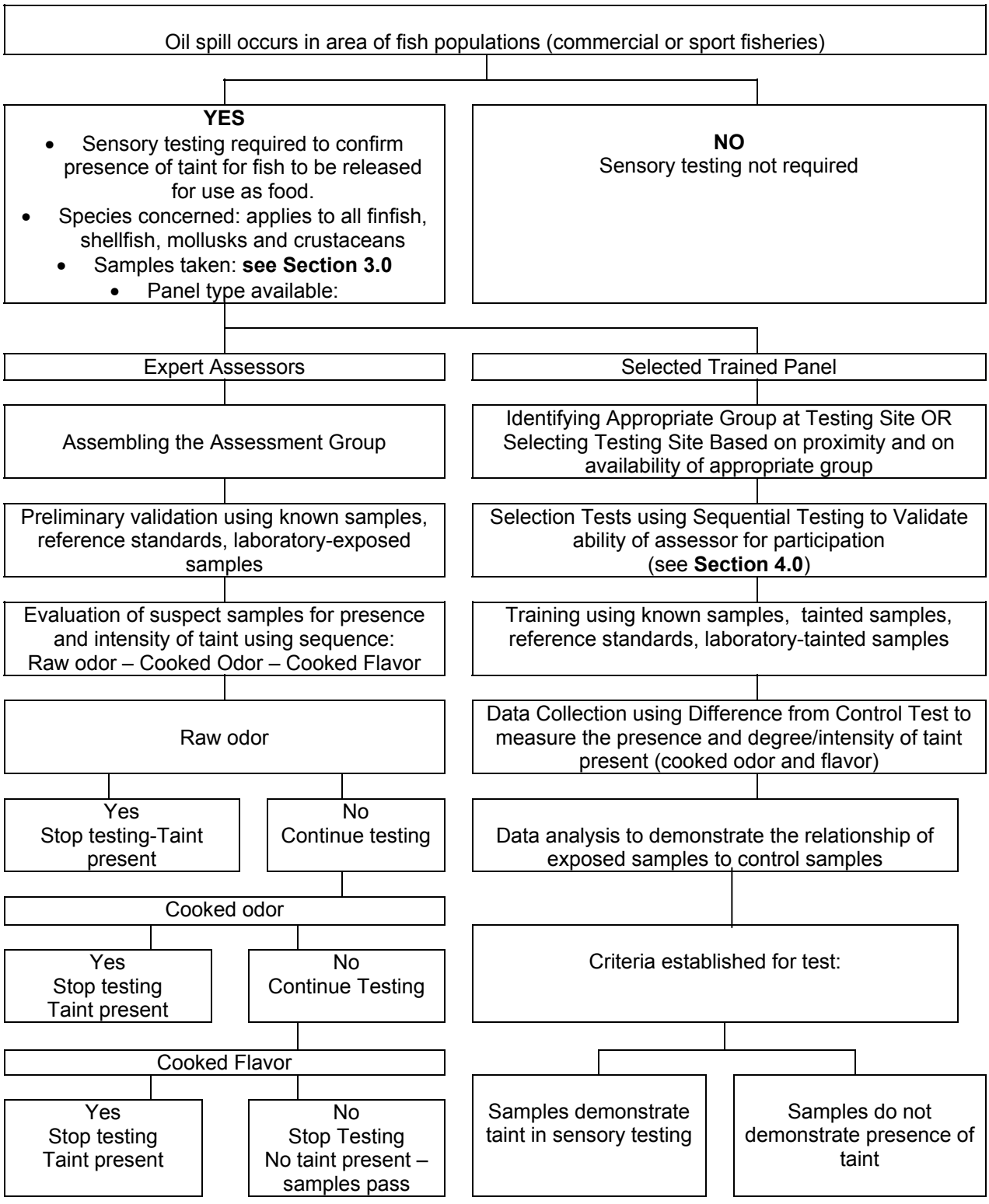


Figure 3. Decision Tree for Sensory Testing Methodology.

4.0
Selecting, Training, and Validating Sensory Assessors

In order to conduct objective sensory analyses of seafood, assessors must be: 1) selected for their ability to perform the sensory task required, 2) trained in the application of the required test method, and 3) monitored (or validated) for their ongoing ability to effectively perform. Assessors are chosen to work within specific test protocols and must be trained to perform within those criteria. The level of training needs vary with the test method, the assessor type, and the form of data analysis that will be used.

In general, the higher the level of training which has been given (as in expert assessors), the less statistical variability in the sensory data and the fewer number of assessors required. Also note – in addition to performance, availability should be a factor in selecting assessors, as they may be required to travel to a laboratory or spill site for several weeks.

4.1 *Expert* assessors

4.1.1 On-site validation

Generally, expert assessors are already employed as seafood sensory analysts and routinely perform overall quality evaluation of seafood products, including taint (Reilly and York 1994), so are already trained. In addition, many government sensory analysts in the US and Canada have received training specifically for petroleum taint in seafood. These individuals should be chosen when possible.

Expert assessors should be re-validated on-site for their ability to perform the test in case the test results are used in litigation. This validation can consist of a series of sequential tests (as would be used for screening panelists for sensory training), and demonstrates that the individuals have the sensory abilities to perceive the sensory attributes for which the samples are being examined, and it serves to re-familiarize or, re-calibrate the assessor.

4.1.1.1 Sequential testing

The groups of tests that fall under the heading of ***sequential testing*** are actually difference tests that are used with pre-set statistical criteria. They allow the decisions

"accept for panel," "reject for panel," or *"continue testing"* to be made via a pre-set graphic representation of the statistical criteria (see **Appendix 6**). The benefits of sequential tests, as described in the sensory literature (Amerine et al. 1965, Munoz et al. 1992; Meilgaard et al. 1999), are that they "economize" on the number of tests needed to *"accept*" or *"reject"* a candidate, yet they also provide a statistical basis for accept/reject decisions. Sequential testing is based on pre-selecting the statistical levels of α and β probabilities for the performance level of the assessors using difference tests such as triangle, duo-trio, and paired comparison-difference. The test is conducted using a minimum of 6 sample sets to begin and continuing until the results from the assessor being tested moves into the accept or reject range of the control chart (see **Appendix 6**).

4.1.1.2 Triangle test

In this type of situation, the triangle test can be used effectively, as there is adequate control over the nature of the treated and control samples. Control charts for these tests and probability levels can be calculated using the methods described in Meilgaard et al. (1999). As mentioned above, sequential testing using the triangle test or other difference tests requires the calculation of control graphs to evaluate the performance of each assessor candidate. The criteria used in calculating the control graphs (see **Appendix 6**) are based on the type of test used and the levels of statistical significance chosen. The recommended levels of statistical significance are α=0.05 and β=0.05.

Samples that can be used for selection of expert assessors include:

- tainted seafood from prior field sampling or laboratory exposures;
- an appropriate model system using either seafood flesh or water as the carrier and appropriate dilutions of critical compounds identified in the petroleum water-soluble fraction;
- concentrations chosen should represent a level of difficulty appropriate for the taint tested.

The recommended procedure is the ***3-alternative forced-choice (AFC) triangle test***. The three samples always include two controls and one treated sample. This

minimizes carry-over effects and increases the chance of being able to detect the different sample (thus increasing the sensitivity of the test).

The sample sets are presented in the triangle test format, and the resulting correct/incorrect responses plotted on the control chart shown in **Appendix 6**. The samples to be used should be standardized according to the concentration range of stimulus that might be expected in the final testing. When the triangle test is being used, the samples used in selection *must* be homogeneous in nature and demonstrate *no other differences* except for the tainting mixture.

4.1.2 Training *expert* assessors

Specific details on training expert assessors in general can be found in the ISO-8586-2 document referenced in **Appendix 1**. For evaluation of seafood for presence petroleum taint, expert assessors are trained with known (tainted and non-tainted) seafood samples. The ballot used in the US is illustrated in **Appendix 4**. The same ballot used for final testing is used for training. All expert assessors must demonstrate that they are evaluating the known samples appropriately. Data are collected and analyzed statistically either by a. the analysis of variance of the taint intensity data or, b. by Fisher's exact test applied to the pass/fail decisions (see **Appendix 11**). Analysis of variance is the preferred method of data analysis. If analysis of variance is used for data analysis, there must **be *no assessor by sample interaction*.**

For expert assessors, there is a 3-tiered approach to evaluating each sample as summarized below and explained in further detail in **Section 7.0**.

The test for ***RAW ODOR*** is sequenced as follows:

1) Coded samples of fish are presented for analysis of the raw odor.
2) Assessors decide whether samples *"pass"* or *"fail"*, based on presence of taint (any presence causes a sample to fail).
3) Intensity of taint is rated on the scale provided, from none (0) to strong (3). Any comments regarding the nature of the taint (descriptors) are also recorded.
4) If taint is detected in the raw-odor test, then testing stops there, if no taint is detected in the raw state, then the sample is cooked.

The test for ***COOKED ODOR*** is sequenced as follows:

1) The sample is cooked following standardized sample-preparation instructions for steaming or microwaving.
2) Odor of the cooked sample is assessed using the same ballot.
3) If taint is detected, then testing stops there, if not, the test proceeds to evaluation of cooked flavor.
4) Intensity of taint is rated on the scale provided, from none (0) to strong (3). Any comments regarding the taint are also recorded.

The test for ***COOKED FLAVOR*** is as follows:

1) If no taint is detected in the cooked-odor test, the flavor of the cooked sample is assessed following the instructions for product testing given in **Section 7.0**.
2) If no taint is found at this point, the sample is considered *"Clear"*.

Further details of expert assessor test instructions and evaluation criteria can be found in **Section 7.2**.

4.1.3 Monitoring *expert* assessors

The test method for monitoring is identical to that used for training. Known, clear samples are included as controls to monitor the performance of the expert assessors. If available, known samples prepared by laboratory exposure or by *spiking* may also be used as controls. Spiking may not produce the same sensory properties as live exposure in a laboratory or real spill situation.

4.2 *Trained* assessors

4.2.1 Selecting assessor trainees

The selection of assessors for a trained sensory panel is conducted using the ***triangle test*** as described above for expert assessors. The selected, trained panel consists of 10 to 15 panelists.

4.2.2 Training assessors

Specific details on training expert assessors in general can be found in the ISO-8586-1 document referenced in **Appendix 1**. For an oil spill situation, assessors can be trained using the ***difference-from-control*** procedure, as shown on the ballot in **Appendix 5**. The control is always a clear sample of the same species, identified by marking it "C" or "Control." All other samples are presented with three-digit codes to mask their identity.

Known samples are used for training (see ballot shown in **Appendix 5**). Panelists are instructed to follow the instructions on the ballot and to use tasting procedures outlined in **Section 7.3**. In training, data from the known samples are collected as blind-coded samples in the test included in the data analysis (using analysis of variance). Assessor performance is measured by the ***main effects*** and the ***assessor by sample interaction*****.** There should be no statistical significance for either of these. Any panelist found to be assessing samples differently from the other selected assessors must be dropped from the panel and replaced by another trained panelist. If a statistician is not available, refer to Post, Mackie, Butler and Larmond, 1991 for a simple description and example of calculation of 1-way or 2-way analysis of variance (i.e. one main effect or 2 main effects).

4.2.3 Monitoring trained assessors

Trained assessors are monitored by including known, clear reference samples presented with the tainted or suspect samples. If available, known samples prepared by laboratory exposure or by *spiking* may also be used as coded samples for monitoring purposes. Again, spiking may not produce the same sensory properties as live exposure in a laboratory or real spill situation.

4.3 Experimental design and data analysis for testing assessors (both expert and trained assessors)

Data collection for taint analysis must allow appropriate statistical methods to be used to interpret the results. Data should include:

- ***descriptive statistics*** of the number of samples and the numbers that demonstrate taint.

- ***statistical analysis*** of the data, including variables such as species, location, panelists, etc.
- ***mechanism for monitoring*** the performance and consistency of the experts and/or trained assessors, for the purpose of demonstrating the effectiveness of the measurement tool.
- ***comparisons*** of samples of affected area *against* control samples.

When possible, the experimental design should be done with the advice of a statistician. Studies should be conducted, as much as possible, as a randomized, complete block design to allow for simple analyses though analysis of variance and for clear presentation of the data, especially when the data will be used as part of litigation. The *main effects* include factors such as species, area, time of sampling, and assessors. Use of the randomized, complete block design allows for 1) interactions to be calculated and 2) assessors to be monitored through both the ***main effects*** and ***sample interactions.***

Data will be collected as numbers decoded from the ballots.

- Ballot for sequential testing using 3-alternative forced-choice triangle test (see **Appendix 3**)
- Ballot for use by expert assessors (see **Appendix 4**)
- Ballot for the difference-from-control test (see **Appendix 5**)

In the case of expert assessors, a pass/fail decision is recorded as *Pass = 0* or *Fail = 1* for raw odor, cooked odor, and/or cooked flavor (see **Appendix 4**). The intensity is also recorded as a value (0, 1, 2, or 3). Because the intensity scale is used as a continuous scale in training, the numbers may be used in data analysis as well as through descriptive statistics.

In the ***difference-from-control*** test (see **Appendix 5**), data are recorded on a sensory scale which represents the perceived difference from the control sample. The difference is measured using either a 6-inch ruler with increments of 0.1 inches, or with a 15-cm ruler with 1-mm increments. For inches, the scale is recorded from 0 (no difference) to 60 in. (extreme difference). For centimeters, the scale is recorded from 0 (no difference) to 150 cm (extreme difference). In both cases, results are reported to the nearest whole number; no decimal fractions are recorded.

4.4 Test systems for assessor selection (both expert and trained assessors)

4.4.1 Model system solution of water-soluble fraction of petroleum

The formulation of chemicals present in the water-soluble fraction of crude oil is presented in **Figure 4**. This formulation, developed by Derek Murray, Canada Department of Fisheries & Oceans (Freshwater Institute, Winnipeg), was first used in 1985. This model system has been used in selecting assessors when data are used in litigation.

Hydrocarbon component	Amount used (mg/L = ppm)	Actual amount used (μL/L)
Benzene	5	5.8
Toluene	5	5.8
ethyl benzene	5	5.8
m-xylene	5	5.8
p-xylene	5	5.8
o-xylene	5	5.8
n-propyl benzene	2	2.33
1,3,5-trimethyl benzene	2	2.33
cumene (isopropyl benzene)	2	2.33
cymene (isopropyl methylbenzene)	2	2.33
Total	38 ppm	

Figure 4. Model system solution of water-soluble fraction of petroleum for testing sensory assessors.

Serial dilutions are prepared at approximately 38, 19, 9.5, 4.75, and 2.375 ppm.

Suggested sets for the triangle test are:

Control: 2 samples, **9.5 ppm:** 1 sample,

Control: 2 samples, **2.375 ppm**: 1 sample,

Control: 2 samples, **4.75 ppm:** 1 sample.

These samples are presented in the ***3-alternative forced-choice (AFC)*** formats of the triangle test. In this case, the sample sets always contain two control samples (clear) and one tainted sample. This allows the taint to be assessed against a clear reference at all times, and makes the test more sensitive in allowing assessors to distinguish the presence of the petroleum tain

4.4.2 Tainted and non-tainted known samples

Laboratory-controlled known tainted ("live") and non-tainted control samples is the most realistic method for selecting assessors. Although this method is very costly and labor intensive, it will provide the most realistic conditions (Poels et al. 1988; Ackman et al. 1991; Davis et al. 1992) and the taint is presented in the actual seafood system. A detailed laboratory exposure procedure (Hufnagle 2000) is included in **Appendix 7**.

4.4.3 Solutions of 1-butanol

An alternative test is based on the use of solutions of 1-butanol (as described in ASTM E544; **Appendix 1**). In this case, the triangle test uses two sets of samples: one in which the control sample is a clear reference, and a second in which the control contains the test compound, to make the test more difficult. This test has been developed for use by York and Zhang (1998) for testing assessors of airborne malodors and may be used for other taint assessments as well.

The test sets are comprised of:

Series 1 [2 clear reference (water) plus one 80-ppm 1-butanol in water]
3 sets each, min [2 clear reference (water) plus one 40-ppm 1-butanol in water]

Series 2 [2 20-ppm 1-butanol in water plus one 80-ppm 1-butanol in water]
3 sets each, min [2 10-ppm 1-butanol in water plus one 40-ppm 1-butanol in water]

Each of these series is presented as separate, sequential testing procedures in which the assessor must pass the first test to proceed to the second. The assessor must pass both procedures to be accepted.

(NOTE: In some cases, it is possible for a candidate to perform poorly in the butanol test, but to perform very well with actual petroleum-based samples). **If at all possible**, model systems outlined in **4.4.1** and **4.4.2** should be chosen.

4.5 Data analysis for evaluating assessor candidates

Data from the testing can be recorded as below, and transposed into a suitable format for analysis of variance through programs such as Microsoft Excel™. For analysis through SAS, a format such as shown in **Appendix 8** is used.

Species	Location	Date	Sample #	Analyst Results		
				1	2	3

4.5.1 Data analysis for difference-from-control test

1) For each sample/code/panelist, measure the response using a 15-cm ruler. Record the response as a measurement in millimeters (to the nearest mm.). The scale is therefore 0 to 150.
2) Record the response in an electronic data table, such as in Excel, or coded appropriately for analysis by SAS or SPSS programming through analysis of variance for difference from the control sample. The control sample will show some readings that will give an indication of the sensory "noise" in the test method.
3) Use the appropriate available program to analyze the data.

4.5.2 Data analysis using SAS

The following is an example of the format for data coding for a randomized complete block design for the difference from control test. Each sample, which is assigned a unique three-digit code, has the following information associated with it:

Spill site	Site 1, Site 2, etc., as identified in sampling plan for the area 1 = …….. 2 = …….. 3 = …….. etc.
Date	Each sampling date and/or time is given a unique number code 1 = …….. 2 = …….. 3 = …….. etc
Species sampled	Each species of seafood sampled 1 = …….. 2 = …….. 3 = …….. etc
Replication	Number of replicate samples tested – each is coded individually 1 = …….. 2 = …….. 3 = …….. etc
Assessor	Unique number assigned to each assessor 1 = (name) 2 = (name) 3 = (name) etc.

This is then coded into a worksheet in a program such as Excel (which can be imported into SAS™ or SPSS™).

Site	Date	Species	Replication	Assessor	Sample code (3-digit)	Reading
1	1	1	1	1	(aaa)	
1	1	1	1	2	(aaa)	
1	1	1	1	3	(aaa)	
1	1	1	1	4	(aaa)	
1	1	1	1	5	(aaa)	
1	1	1	1	6	(aaa)	
1	1	1	1	7	(aaa)	
1	1	1	1	8	(aaa)	
1	1	1	1	9	(aaa)	
1	1	1	1	10	(aaa)	
1	1	1	1	1	(bbb)	
1	1	1	1	2	(bbb)	
1	1	1	1	3	(bbb)	
1	1	1	1	4	(bbb)	
1	1	1	1	5	(bbb)	
1	1	1	1	6	(bbb)	
1	1	1	1	7	(bbb)	
1	1	1	1	8	(bbb)	
1	1	1	1	9	(bbb)	
1	1	1	1	10	(bbb)	
etc.	etc.	etc.	etc.	etc.	etc.	etc.

4.5.3 Data Analysis using Microsoft Excel™

Examples of data recording for analysis using the **Data Analysis** subroutine under **Tools**:

***One*-way ANOVA**

***Two*-way ANOVA** *with* Replication

***Two*-way ANOVA** *without* Replication

Only simple ANOVAs are possible with Excel. If more than two main effects are tested, SAS™ or SPSS™ or their equivalent must be used.

5.0
Facility Requirements for Sensory Evaluation of Seafood

5.1 Maintaining a neutral environment

The facility in which objective sensory evaluation is conducted is an essential component of sensory test protocols. Sensory testing generally requires a controlled neutral environment in which samples can be evaluated for their intrinsic attributes, here, the possible presence and intensity of taint from exposure to petrochemicals. The testing environment *must not* interfere with or influence the sensory test. Both ASTM and ISO provide excellent guidelines for facility design (see Appendix 1).

Principle of facility-design requirements:

A special test room or area in which to conduct sensory evaluations under constant controlled conditions with a minimum of distractions.

To control the effects from introductions of physiological or psychological errors into human assessments, e.g., background odors causing adaptation and reduced sensitivity.

Components of the neutral environment include:

- lighting of appropriate quality and intensity for the assessments,
- ventilation that is appropriate and adequate to remove odors given off by the samples during testing, and
- freedom from distractions.

Another important consideration is ease of sanitation and the use of products that do not add odors of their own into the test area (odor-free soaps, etc.) Various publications describe the construction of sensory facilities, including ASTM STP913 Physical Requirement Guidelines for Sensory Evaluation Laboratories (see **Appendix 1**).

In evaluating seafood following an oil spill, consideration must be given to the choice of testing locations, as well as to field vs. laboratory testing. It is important to also consider chain of custody during the handling, preparation and evaluation of samples (see **Section 6.1**).

5.2 Sensory testing at the spill site

Field-testing involves actually bringing the assessors to the site where samples are harvested for sensory evaluation. This approach has several *disadvantages* following an oil spill:

- Lack of control over ambient conditions (e.g., air temperature and humidity, provision of appropriate shelter).
- Interference from and adaptation to, environmental odors (e.g., spilled oil, fuel on the boat or ship; rigging, nets, etc.).
- Exposure to distractions (e.g., cleanup personnel, equipment noise, media presence, etc.).

When expert sensory assessors were tested in England on their ability to conduct quality evaluations of products in a field (market) setting, as well as in a laboratory, their evaluations were shown to be accurate in both settings (Aust et al. 1985). However, because of ambient conditions at the oil-spill site that could compromise the objectivity of the testing—and the likelihood that the sensory data on seafood taint would be a component of possible legal actions—we recommend that all sensory assessments relative to oil spills be conducted in an appropriate laboratory setting where the testing environment cannot be challenged.

5.3 Sensory testing in the laboratory

An appropriately designed laboratory, preferably one specifically designed for sensory testing or one that can be converted as such, is the best choice. The key is providing adequate equipment and space for sample storage, preparation, sample evaluation, and data analysis.

Another concern during sensory testing of seafood following an oil spill is the presence of other interested parties (such as media, industry representatives, fishers, etc.), all with a vested interest in the results of the sensory tests. It is essential that they not be allowed to interfere in the assessors concentration on their tasks on the sensory tests; i.e., they should be kept out of the sensory testing room when assessors are evaluating seafood samples.

5.3.1 Sample-preparation area in the laboratory

It is critical that the sample *preparation* area be physically separate from the sample *evaluation* area. The preparation area is used for the storage and handling of seafood samples and for the preparation of samples for sensory evaluation (in the test area). The facility should be constructed so as to comply with the requirements of good manufacturing practices for the design and construction of fishery facilities, and all equipment used in the area must also comply with the requirements for equipment used in fish-processing. The facility design must also be appropriate for a sensory evaluation laboratory, e.g., the rooms must be designed so that odors from sample preparation and cooking do not transfer into the sensory evaluation area.

The **sample-preparation area** should provide:

- ***refrigerators and freezers*** adequate for the temporary storage of chilled and frozen seafood, and for the freezing of samples. Freezers must have < -20º C temperature capability.
- ***storage facility*** for glassware used for sample presentation for evaluation
- ***tables and benches*** (one or more) for the preliminary handling and inspection of batches of material,
- ***table*** (at least one) suitable for the wet-processing operation of filleting of fish, handling of crustaceans and molluscs, preparation of blended samples (BIFs), etc.
- ***counter-space*** suitable for the preparation and coding of final samples for serving in the evaluation area,
- ***large sink*** (at least one) for thawing samples and for washing containers, utensils and equipment used in the preparation and evaluation areas.
- ***cooking facilities*** (microwave ovens, steamers, etc.) for the cooking of samples as needed during evaluation. Stoves should be electric (gas or propane is another source of potential off-odors in the background air).
- ***equipment*** including:
    - stainless-steel trays, medium (~50x40 cm) and large (~70x60 cm)
    - filleting boards, filleting knives, sharpening stone and steel
    - plastic or metal containers for fish offal
    - containers for other rubbish

- utensils and materials for cleaning and disinfecting premises, equipment, and utensils
- glass or ceramic baking dishes with lids (not plastic), suitable capacity for holding samples of finfish, molluscs, and crustaceans
- digital thermometer, range -50 to 300º F (-45 to 149º C)
- electronic balance
- assorted stainless steel kitchen utensils, knives, serving spoons, etc.

If possible, all cooking equipment and utensils should be kept separate for the *control* and *suspect* samples. If functionally, it is not possible to keep the two groups of equipment separate, everything must be fully cleaned in between sample sets using unscented soap and several rinses in odor free water. **Cooking equipment** such as microwave ovens and steam tables ***must*** be kept separate, as it is not possible to completely clean these in between sample sets.

5.3.2 Sensory-evaluation (testing) area in the laboratory

This area is to be used ***only*** for sensory evaluation (testing) of raw and cooked seafood samples delivered from the sample-preparation area. There must be ***no*** preparation of products in this area. The surroundings should be sensory-neutral (e.g. color, odor) and free from distraction, so that the personnel involved in the sensory test can concentrate on evaluating the products.

This area should be constructed and furnished so that it can be maintained in a clean and hygienic state. However, because there should be no seafood preparation in the area, it need not comply with requirements for seafood-processing facilities. It should, however, comply with the guidelines for food preparation areas in catering establishments and with ASTM STP 913 (see **Appendix 1**).

- ***Floors*** should be finished with a seamless, waterproof coating.
- ***Wall surfaces*** should be smooth and painted white or very light, neutral grey or beige, using washable paint; or finished in tile or odor free plastic sheet material in white or neutral grey or beige.
- ***Workbenches*** should be constructed of, or finished in, impervious material that can be sanitized and disinfected. Any joint between benches and walls should be sealed with waterproof mastic. Benches may incorporate cupboards and

drawers, and cupboards may be fitted to the walls above the benches. Bench tops should be white or pale neutral grey or beige.

- ***Lighting*** should be from fluorescent tubes providing about 1000 lux/m^2 (92.9 or approximately 100 foot-candles), of color-matching quality (5000º K or 6000-6500º K), and with a color-rendering index of greater than 90%.
- ***Air handling*** equipment to create positive pressure to facilitate removal of odors. Supplementary electronic air purifiers with activated carbon filters may also be used.
- ***Room temperature*** should be controlled to between 68 to 75º F (20 to 24º C), and ***relative humidity*** is 45%, so should be within that range.
- ***Washbasin or sink*** supplied with hot and cold water and odor-free hand soap.
- ***Odor-free drinking water*** should be available for rinsing during evaluation sessions, either filtered water from the laboratory water supply, or bottled water
- ***Small equipment*** including
  - warming plates to keep cooked samples at serving temperature
  - cutlery (forks, spoons, table knives)
  - glass jugs and/or beakers for water or other rinsing materials

The sensory-evaluation area is arranged in one of two different ways, depending on the *type of sensory evaluation* being conducted and the *type of assessors* being used (expert or trained).

5.3.3 Workshop facility

Expert assessors usually work in a ***workshop***-type facility, where samples are laid out in individual stations and the assessors move from one sample to another to perform the evaluations. This area must meet the requirements for lighting and ventilation (odor control), minimize distractions, and ease sanitation.

A workshop facility consists of large areas of benches (e.g., counters or stainless-steel tables on casters for easy mobility) on which are movable dividers: three-sided units either hinged to the wall or free-sanding. Dividers are constructed of non-porous, easily cleaned materials, as are the countertops. Individual samples are placed in each of the divided spaces, with assessors moving from one sample to another. If dividers are not available, assessors may work on samples in the same area if they are

positioned far apart and are not facing one another. This type of facility is designed to accommodate larger sample sizes and to facilitate movement of assessors so that all assessors can examine every sample (see **Section 6.4.2**).

5.3.4 Booth facility

Trained assessors usually work in a traditional ***booth*** facility in which the assessors are stationary in booths and the laboratory technicians bring the samples to them. In this facility, individual booths are provided to control the test conditions and provide a comfortable evaluation environment. Smaller, individual samples or sample sets are presented to each assessor for evaluation, and data are collected from the test ballots (see **Appendix 5** for ballot sample). In this type of facility, each person receives his or her own individual sample or set of samples. The individual samples are made homogenous in nature by preparing small aliquots from multiple blended organisms (see **Section 6.1.2.2**). Criteria for construction of panel booths are given in ASTM STP913 (see **Appendix 1**) and generally include:

- ***Enough booths*** for the size of the sensory panel (panel sessions are scheduled to accommodate individual assessors).
- ***Countertop and walls*** are constructed of non-porous material and are neutral in color and durable.
- **Booths** are generally ***27 to 32 inches wide*** (~69 to 82 cm) to allow adequate workspace (for tray, ballot, judge), and are fitted with a hatchway door for sample presentation and removal.
- Booths may be ***computer-equipped*** for data acquisition during sensory testing
- ***Dividers*** extend beyond the countertop to minimize distraction during panel sessions.
- ***Lighting*** should be even and of the quality and quantity specified above
- Booths are equipped with ***adjustable chairs*** of appropriate height and having casters for ease of mobility.
- Booths may be equipped with ***rinse sinks*** (although not generally recommended because of specialized cleaning needs and potential problems with odor control).

6.0
Sample Handling, Preparation, and Presentation in the Laboratory

6.1 Personnel involved in sample handling, preparation, and presentation

- Assessors who will evaluate the samples for presence of taint should ***not*** handle or help prepare the samples *at any stage* before the test.
- The individuals who will be responsible for preparing samples must be trained and are usually referred to as ***facilitators*** or ***technicians***. Individuals who prepare the samples can ***not*** participate as assessors. These individuals should follow the guidelines for odor assessment panels and refrain from using any scented personal care products in any form (especially perfumes, after-shaves, etc.). These individuals must not smoke, must have clean, odor-free hands, and must wear clean clothing that is odor-free. Lab coats or aprons should be worn only in the laboratory setting.

6.2 Secure handling of samples

To ensure ***chain of custody***, samples are to be handled in a secure manner at all times, and their inherent sensory characteristics are to be preserved (see also **Section 3.0**).

6.2.1 Sample receiving

- Keep detailed records of the date and amount of sample received, sample condition, labeling and codes present, storage procedures, and names of personnel handling the samples (see **Appendix 10** for an example of a chain of custody form and detailed instructions). Notes should be taken in a hardbound notebook specifically designated for this project. If control samples were collected, make sure that they are clearly labeled.
- Upon receipt, unpack samples immediately and check for any physical damage. Have a trained sensory assessor check to see if any quality changes have occurred. Samples should not be decomposed.

- If sensory testing is to be done immediately and can meet the 24-hour time window, *re-ice and refrigerate immediately* (see storage procedures in **Section 6.2.3** below).
- If samples are shipped frozen, check to see that an adequate frozen state was maintained. If any samples have thawed, separate these from the others and label. Have a trained inspector or expert assessor check for decomposition).
- After unpacking, record sample information and place immediately on ice or in frozen storage as described in **Section 6.2.3** below.

6.2.2 General principles of sample preparation

The goal is to prepare samples for presentation to the assessors without causing any quality changes or imparting any off odors/flavors due to handling or cooking.

- Personnel who will be preparing the samples should perform preliminary ***sample-preparation trials*** to determine and standardize thawing and cooking times.
- If samples can be tested within 24 hours of capture, freezing is not necessary as long as samples are kept adequately cold (4° C or below).
- ***Only*** clean glassware, aluminum foil or stainless steel should be allowed to come in contact with the samples ***at any time***. Any other materials, particularly plastic packaging material, could impart, or be suspected of imparting *off* odors and/or flavors. Make sure that all thaw water is clean and odor-free.
- If control samples are incorporated into the test design, these samples must be handled, stored, prepared, and presented in ***exactly the same*** manner as the suspect samples.
- If chemistry is to be performed, work with the chemists to determine the numbers and volume of sample that they will need. Chemistry samples are to be handled in exactly the same manner as sensory samples. If correlations will be performed on the chemistry and sensory data, measurements should be taken ***from the same sample***, as fish do not taint uniformly.
- An experimental design must be determined before sample preparation begins (see **Section 9.0**). Prepare enough samples to ensure enough for each treatment and replication. An expected flesh recovery table is included in **Section *2.7.2***.

- In addition to the muscle tissue, it is common in certain societies to consume other parts of finfish and crustaceans such as the roe, tamale, or certain organs. In this case, all parts normally consumed should be evaluated for tainting. For pooled, blended samples, enzyme-containing organs should be separated from the muscle tissue and evaluated separately.
- ***Nothing*** may be added to change the odor or flavor of the edible tissue. This includes condiments, such as salt.
- For ***expert assessors***, either intact tissue portions or whole organisms will be prepared for testing.
- For ***trained assessors***, individual foil packet samples (BIFs) will be prepared from the normally consumed portions of pooled multiple organisms.

From this point forward this section is organized according to the 2 sample preparation styles ("intact organisms" and "pooled, blended organisms") as necessitated by assessor and test type and described above. A full description of each sample style can be found later in this section.

Table 2. Summary flowchart of sample preparation and presentation.

Intact single organism **samples for** ***expert*** **assessors**	***Pooled, blended multi-organism*** **samples for** ***trained*** **assessors**
Single organisms, or parts thereof, presented in covered *glassware*	Multiple organisms, blended, portioned, and presented in individual *foil packets*. (BIFs)
One blind-coded organism presented at each station; assessors move from station to station.	Stationary panelists are presented with multiple blind-coded samples at each station.
All assessors evaluate each sample.	One assessor evaluates own set of foil-packet samples.
Vacuum-sealed samples thawed under refrigeration or in cool, running water.	*Frozen foil* packets thawed under refrigeration or cooked from frozen state.
Raw samples presented first for odor evaluation in covered glassware at ambient temperature.	Samples evaluated in *cooked state only*. For odor, then flavor.
Samples *microwaved* in covered glassware to internal temperature of 160° F and placed on warming trays back at stations in evaluation lab.	Samples *steam-cooked* 7 mins. if thawed, or 10 mins. if frozen. Transfer one set for each panelist to warming trays in evaluation booths.
In pre-determined order, assessors evaluate odor of all samples found negative for taint in the raw odor evaluation. Assessors then *taste* all samples found negative for taint in the raw and cooked odor evaluation steps (see **Section 7.2**).	In predetermined order, assessors evaluate *odor* of all samples first, re-folding the packets as they go. Assessors then *taste* all samples found negative for taint by odor evaluation (see **Section 7.3**).

6.2.2.1 Intact organism samples

Intact organism samples are usually presented to expert assessors, and are presented raw first, then cooked as outlined below in Section 6.3.1.

- ***Finfish*** Remove edible muscle tissue by filleting. If the species is commonly sold and consumed skinless in the marketplace, remove the skin. If the species is usually sold skin-on, leave the skin on the fillets but remove scales first from the entire fish. Keep cool if testing is to be done immediately, or wrap each fillet in heavy-duty aluminum foil, vacuum-seal and freeze at < -20º C.
- ***Bivalves or Mollusks*** Rinse live organisms in shell, organize into sample units of 3 to 6 (see **Section 2.0**), and keep cool if testing is to be done immediately. If samples are to be tested later, wrap 3 to 6 live, in shell organisms together in heavy-duty aluminum foil, vacuum-seal and freeze at < -20º C.
- ***Crustaceans*** Keep cool and moist if testing is to be done immediately. If testing is to be done later, place live organisms in freezer until all movement stops, then remove, wrap with heavy-duty aluminum foil, vacuum-seal and freeze at < -20º C.

6.2.2.2 Pooled, blended samples

Pooled, blended samples are usually presented to ***trained assessors***. To prepare pooled samples, the edible parts of multiple organisms are blended, divided into 20-gram aliquots and sealed in individual foil packets as outlined below. These samples are evaluated in the cooked state only.

- ***At least 6 organisms*** are recommended for pooling per sample to minimize the effects of natural flavor differences among organisms; however, a minimum of three organisms can be acceptable if samples numbers are limited or the individual organisms are large.
- ***Standard measurements***, such as size, weight, length and sex (if possible), must be recorded before pooling.
- ***Detailed-harvesting records*** must be kept. Pooled samples must be from the same harvest location.
- Sample preparation should result in ***uniform samples***.
- Keep seafood tissue ***cool*** at all times.

- If preparing samples from frozen seafood, ***thaw*** samples under refrigeration only enough to handle.
- ***Finfish*** Blend edible tissue from 6 fish just long enough to homogenize.
- ***Bivalves*** Remove the meats from six organisms and blend.
- ***Crustaceans*** If any organs or other body tissues are commonly eaten other than muscle tissue, these must also be evaluated for taint, but kept distinct and evaluated separately. First, separate commonly consumed sections from six organisms, then pool, blend, and freeze. (For example, for lobsters, pool, blend and prepare individual samples with the muscle tissue from six organisms, separately blend the tamale from six, etc.).

6.2.3 Storing samples

All frozen samples must be protected from dehydration and oxidation either by vacuum sealing or through a combination of glazing and airtight wrapping. The sample must be wrapped in heavy-duty aluminum foil before placement into any plastic vacuum bag or plastic wrapping.

- ***Freeze samples quickly*** and store in a freezer at -20° F or below, keeping samples from different sources separate and clearly labeled. The freezer must be clean and odor-free.
- For ***security*** purposes, the freezer should have a lock, or samples must be stored in a locked container or compartment in the freezer. The number of personnel with access to the samples must be limited and recorded.
- The freezer should be equipped with a ***temperature alarm system*** and the freezer temperature should be periodically monitored.

6.3 Sample preparation for sensory testing

Prior to each day of sensory testing, identify and gather the samples that will be tested on the following day and organize by sessions.

All sample preparation will be done in the preparation area before being brought to the evaluation area of the laboratory.

6.3.1 Intact single/multiple organism samples

As stated above, this type of sample consists of whole or edible parts from single or multiple animals and is usually presented to expert assessors. These samples will be evaluated for raw odor, then cooked and evaluated for odor and flavor. Samples should be presented as they are normally purchased and consumed in the marketplace. This will vary with species and region (i.e., in the United States, finfish is usually purchased and prepared in the filleted state, either skinless or skin-on, depending on the species, while in many other countries, it is usually purchased and prepared whole or dressed). Raw samples should be presented at ambient temperature. These samples should be presented to assessors as soon as possible and not allowed to remain at ambient temperature, as this will cause quality changes.

- ***Frozen* samples** must be thawed in their vacuum-sealed packaging, either under refrigeration overnight, or in cool running water before the session. Allow them to reach room temperature before presenting to the assessors.
- ***Chilled* (never been frozen) samples** are to reach room temperature before testing.
- ***Raw* samples** must remain, after thawing, at refrigeration temperature and in their vacuum sealed packages until approximately 1 hour before the test, at which time they are brought up to ambient temperature in covered glassware. After initial raw odor evaluation by the assessors, the same samples will be cooked by the facilitators and placed back in the evaluation laboratory.
- ***Cooked* samples** are to be heated to an internal temperature of 160° F. Appropriate cooking methods include steaming or microwaving in glassware. Samples must be cooked uniformly, and equipment must be calibrated. Perform cooking trials for each species, each type of cooking vessel, and each piece of cooking and warming equipment. Note on each piece of equipment the specific cooking and/or warming instructions required bringing samples to an internal temperature of 160° F for each species. Samples are to be kept warm and maintained at a temperature of 140–150° F with electric warming trays at the booths or stations in the sensory evaluation laboratory.

6.3.2 Blended, pooled samples

- ***Blended, individual foil packets (BIFs)*** can be cooked either from a frozen state, or from a thawed state. Samples should be uniform in appearance, amount, and temperature. To minimize browning of the samples, steaming is preferred.
- **To *cook*,** arrange foil packets on a rack in a steamer, allowing enough room for steam to circulate. Cook for about 7 minutes if thawed, or 10 to 12 minutes if frozen (a trial run should be conducted ahead to determine exact times). Transfer cooked samples via stainless-steel tongs to electric warming trays at the booths in the evaluation laboratory to maintain a temperature of 140 to 150° F. Do not hold samples on warming trays longer than 15 minutes.

6.4 Sample presentation in the evaluation area

6.4.1 Preliminary preparations

A set of 3-digit random numbers for blind-coding the samples and a template for randomized presentation order should be generated ahead of time.

6.4.2 Sample placement and timing

- The sample design must include randomized order of presentation of samples within a session (see **Section 6.5** below).
- All coded samples should be placed at booths or stations in the evaluation laboratory by the facilitators before the assessors enter the area.
- Samples should be presented in sessions (or sets) with a 15 to 20 minute break in-between.
- Assessors should generally evaluate a maximum of eight samples per session to minimize sensory fatigue and carry-over effects.
- For ***trained*** assessors, *multiple samples* (~ 8) are placed at each booth or station where the panelist remains stationary. With ***expert*** assessors, each *intact organism sample* is presented at a booth or station and the assessors rotate among them. All assessors evaluate all the samples.

- For ***trained*** assessors, the order of sample presentation within a session must be randomized. With ***expert*** assessors, order is randomized as they rotate among the stations.
- ***Intact organism samples*** should be presented in glassware large enough to allow volatiles to equilibrate in the headspace for sniffing. The ratio should be about 1/3 sample to 2/3 headspace. Samples must be covered at all times to allow volatiles to accumulate in the headspace, except for when an assessor is actually engaged in smelling or tasting.

6.4.3 Presentation of intact organism samples

All samples are random coded in microwave-safe covered glassware (such as Pyrex™) and placed in pre-determined positions in the evaluation area in the raw state first before any cooked evaluation occurs. After the assessors evaluate all the raw samples, the samples are removed from the evaluation area, cooked, and placed back at their original stations in the evaluation area on warming trays. The assessors should leave the evaluation area for a break during this time.

- ***Raw evaluation*** Raw samples are presented in coded, covered glassware. Assessors will follow the raw odor evaluation protocol as stated in **Section 7.2.3**.
- ***Cooked evaluation*** Facilitators will remove samples from their booths or stations and cook according to **Section 6.3.1**. Facilitators then place cooked samples back in their original positions in the sensory lab. Samples should remain in their covered glass container at all times. Samples must be placed on warming trays that have been set to maintain an internal sample temperature of 145–150° F. Assessors will follow the cooked odor and flavor evaluation protocol for *expert* assessors, as stated in **Section 7.2.3**.

6.4.4 Presentation of pooled, blended samples

Facilitators place multiple, cooked and coded foil packets, in order, on a warming tray at each booth or station. Order of presentation is usually left to right, front to back. Assessors will evaluate the samples following the protocol for *trained* assessors, as stated in **Section 7.3**.

6.5 Assigning random sample-presentation sequences

Randomizing sequences for sample presentation should include:

1) ***Random order of sample presentation among assessors at each session*.** Within the set of samples presented at one session, randomize the order of presentation such that no two assessors get samples in the same order. This is easily done with a random-number table described in **Appendix 8**. This can be done for both expert and trained sensory assessors.
2) ***Random order of sets of samples presented to each assessor at each session.*** In selecting panels, such as when using the triangle test, the sets of samples are randomized for each assessor, and the sequence of samples within each set of three is also randomized. This requires careful attention to the randomization process and careful record keeping for sample presentation.
3) ***Random presentation of sets of samples over all sessions.*** In a larger study and when using trained assessors, by order of presentation over all sessions (when this is possible). This occurs when all of the samples have been prepared ahead of time (as for the BIFs), and individual sets can be drawn for each panelist. This is not usually possible for expert assessors, as all must examine the same sample within the same short time period.
4) ***Exceptions*** There are certain exceptions to the random order of samples, the most important one being ***threshold*** studies which require ascending or descending series of concentrations. In this case, blank samples are inserted at the beginning and within each series, with the number of blanks varying to minimize predictability.

Detailed instructions on the use of random codes and generating random numbers, and randomizing samples are included in **Appendix 8**.

7.0
Sensory Evaluation Protocols

7.1 General sensory testing procedures

The following points are common to all of the sensory test methods described in this document.

1) Do not allow participants to taste any seafood that has died or is suspect of dying as a result of the oil spill.
2) Before entering the evaluation room, the assessors should wash their hands with odorless soap and dry them with low odor (white) paper towels to remove any trace of residue. Assessors must wear clean, odor-free clothing and refrain from using any scented personal care products. Assessors may not smoke before or during testing. Assessors may not wear clothing that has been exposed to cigarette smoke, including lab coats, etc.
3) Assessors must not smoke during the test.
4) During testing sessions, assessors should avoid or minimize touching samples. They should clean hands of any sample residue ***between each sample*** using unscented soap or, if sinks are not available, low-odor paper towels dipped in odor-free water. This is to prevent cross-contamination of samples and physical carry-over of any stimuli.
5) If flavor is evaluated, participants must adhere to strict rinsing procedures and must expectorate all samples.
6) Rinsing between samples using neutral materials is standard practice during sensory evaluation sessions. The purpose of rinsing is to prevent the carry-over of stimuli from one sample to the next. Odor-free water (e.g., distilled or filtered) is recommended along with unsalted soda crackers. When crackers are used, they must be followed by a water rinse to remove any residue from the mouth. It has been found that using odor-free water heated to 50º C is useful in removing flavors between samples. In ASTM D3696-95 (which has been superceded by E-18 1096), the recommendation is still given to use a dilute lemon juice rinse during panel sessions. ***Do not use lemon juice rinse!*** Lemon juice can interfere with low levels of sourness in the samples.

7) Human assessors are susceptible to fatigue and adaptation to petroleum odors/flavors that may diminish their ability to detect taint. Samples should be arranged in sets with maximum number in each session, and assessors must be given a break between sets (see **Section 6.4.2**). Assessors must also take sufficient time between samples within a session.
8) Time-out between samples is required to avoid carry-over of odor/flavors from the previous sample. A minimum of 1 minute should be taken between samples within a session, although a longer time may be needed in specific testing conditions. Assessor fatigue is minimized by rinsing and by limiting the number of samples that are analyzed at each session. The number that can be tested will be established during training. Generally speaking, if there is a strong carry-over effect, 1 to 3 treatments with appropriate rinsing may be the limit for number of samples per session. If carry-over effects are minimal, a larger number of samples can be evaluated, with appropriate rinsing. We have found that expert assessors generally feel that eight to 10 per session is the maximum.
9) Panel methodology for the analysis of taint includes the use of *warm-up* samples at the beginning of each test session. This allows the sensory systems to *re-experience* the sensory attributes that will be evaluated before the actual testing begins. Although data may be collected on these warm-up samples, they are not used in the final analysis.
10) Assessors should be familiar with all instructions ***before*** the test.

The following sections are divided according to protocols for *expert* assessors (as defined in **Section 3.5.2.1**) and for *trained* assessors (as defined in **Section 3.5.2.2**).

7.2 General instructions for *expert assessors*

7.2.1 *Before* testing

- Assessors must wear clean, odor-free clothing.
- No extraneous scents are allowed, e.g., perfume, after-shave, or breath mints.
- Assessors should not eat or drink 1/2 hour before sensory testing.

- Assessors should avoid highly spiced foods the day before and the day of the test.
- Assessors may not smoke before or during the test, or wear clothing smelling of cigarette smoke.
- Assessors must wash their hands with unscented cleanser and odor-free water when they enter lab.
- Assessors must cleanse their mouth by rinsing either with odor-free water or by chewing an unsalted cracker, then rinsing several times with odor-free water.
- The facilitator must review the ballot and test procedure with the assessors.
- Assessors should evaluate a few warm-up samples at the start of each day of testing.

7.2.2 *During* testing

- Assessors must avoid hand contact with samples by using a knife, fork, or tongs to manipulate the sample.
- Assessors should wash hands with unscented soap between samples.
- To sniff or taste the sample, assessors should slide the glass covers off just a slight distance, then quickly slide the cover back. This retains as many volatiles as possible within the glassware for other assessors.
- Assessors should wait at least 1 minute between each sample within a set, taking longer if necessary.
- Assessors should wait at least 15 minutes between sample sets.
- Assessors should evaluate a maximum of 8 to 10 samples within a set.
- For odor evaluation, assessors should take two or three short *shallow* sniffs (sometimes called "bunny" sniffs) and standardize the distance from their nose to the samples.
- Assessors should cleanse their nose between samples by sniffing the back of the hand or arm, or the headspace over a glass of odor-free water.
- For flavor evaluation, assessors must cleanse their mouth between each sample by chewing an unsalted cracker and rinsing with odor-free water. Assessors should standardize the amount of sample placed in the mouth. ***All samples must be expectorated!***

7.2.3 Evaluation criteria

The following 3-tiered procedure is recommended to minimize sensory fatigue and carry-over effects (see **Figure 5**).

1) Evaluate the odor of the ***RAW*** sample for the presence of taint. If taint *is* detected: ***STOP—Sample fails.*** If taint *is not* detected, proceed to ***Step 2.***
2) Evaluate the *odor* of the ***COOKED*** sample for the presence of taint. If taint *is* detected: ***STOP—Sample fails***. If taint *is not* detected, proceed to ***Step 3***.
3) *Taste* the ***COOKED*** sample for the presence of taint. If taint *is* detected: ***STOP—Sample fails***. If taint *is not* detected: ***Sample passes***.

Using this the 3-tier evaluation procedure described above, assessors must evaluate the ***raw odor*** of ***all*** the samples presented in the set first, then proceed to evaluate the ***cooked odor*** of ***all*** the samples found ***negative*** for taint by raw odor, then proceed to evaluate the ***flavor*** of ***all*** the cooked samples that were found ***negative*** for taint by raw and cooked odor. The principle behind this procedure is: if taint is detected in the raw odor of a sample, concentrations are usually well above threshold. If the same sample were cooked and evaluated, the perceived intensity would only be more intense and the assessor would be exposed to relatively high concentrations (of taint) that would add to fatigue and carry-over. The same is true for cooked ***odor*** vs. cooked ***flavor***; if the assessor perceives taint in the cooked odor, it would only become more pronounced in the flavor. Rinsing taint from the mouth is more difficult.

7.2.4 Evaluation ballots

- Expert assessors who have had some descriptive training should use ballots that allow for recording both quantitative and qualitative information. Ballots of this nature usually incorporate a type of category scale with an area to record descriptors. An example is illustrated in **Appendix 4**.
- Because expert assessors are intimately familiar with the seafood products they are assessing (see **Section 8.1.1**), they can disregard “normal” sensory attributes and focus on contamination. Expert assessors should use the ballot to record only the presence and intensity ***of petroleum contamination*** and to provide descriptors of that contamination.

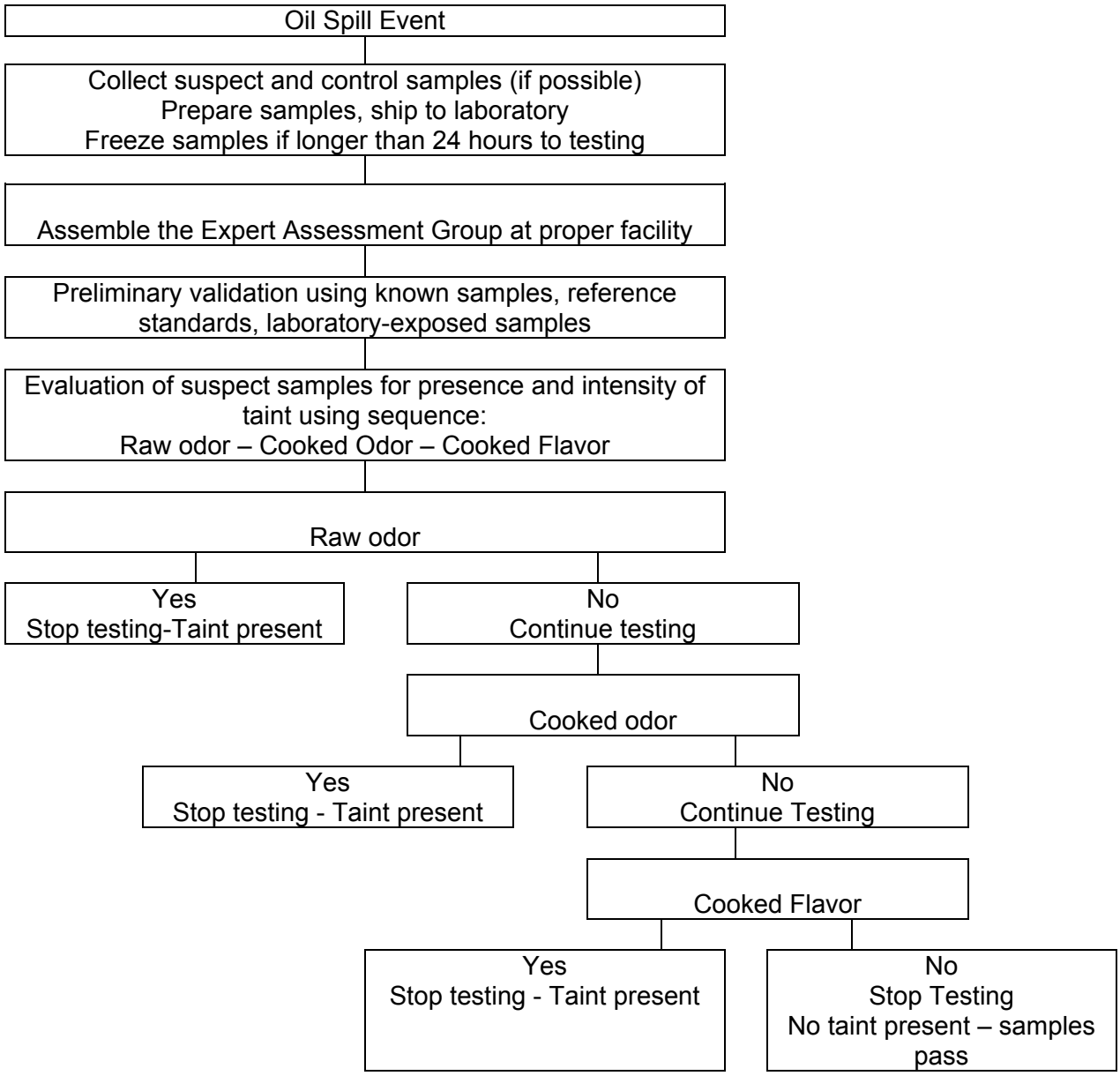


Figure 5. Sensory decision tree for use by *expert* assessors in evaluating petroleum taint.

7.3 General instructions for *trained* assessors

** Assessors should be familiar with these instructions before the test.

A panel of trained assessors will evaluate 20-g packets of pooled and blended seafood samples. The panelists' instructions for handling blended sample packets during evaluation sessions include:

- Open packets by tearing off one folded end and down one side of the foil.

- Evaluate the odor of the samples first, then close the packet temporarily.
- If no petroleum contamination is detected, or if it is of such a slight intensity as to be questionable, samples must be tasted.
- To taste, divide the sample in half and do the assessment. Use second half as needed for re-tasting.
- Cleanse mouth after each sample set.

7.3.1 *Before* testing

- Panelists must wear clean, odor-free clothing.
- No extraneous odors or scents should interfere, such as perfume, after-shave, or breath mints.
- No eating or drinking 1/2 hour before sensory testing.
- Panelists should avoid highly spiced foods the day before and the day of the test.
- No smoking is allowed or during the test.
- Panelists must wash hands with unscented cleanser and odor-free water when entering the lab.
- Facilitator must review the ballot and the test procedure with the panelists.
- Panelists should evaluate a few warm-up samples.

7.3.2 *During* testing

- All samples will be evaluated for odor before tasting any samples.
- Packets are re-folded after odor evaluation to retain volatiles.
- Hand contact with samples must be avoided or kept to a minimum.
- If contact with sample is made, hands should be washed with unscented soap and/or rinsed with odor-free water before the next sample.
- Panelists should wait at least 1 minute between samples ***within*** a set, or longer if felt necessary.
- Panelists should wait at least 15 minutes ***between*** sample sets.
- Panelists should evaluate a maximum of eight samples within a set to minimize fatigue and carry-over effects.
- For odor evaluation, panelists should take two or three short *shallow* sniffs (sometimes called “bunny” sniffs) and should standardize the distance from their

nose to the sample and standardize the number and duration of sniffs. Each foil packet must be folded back up to retain aromatics for the tasting phase.

- Assessors should cleanse their noses between samples by sniffing the back of the hand or the headspace over a cup of odor-free water.
- For flavor evaluation, assessors must begin by cleansing their mouth by chewing an unsalted cracker and rinsing with odor-free water, repeating this procedure between each sample. The amount of sample placed in the mouth should be standardized. **All samples must be expectorated!**

7.3.3 Evaluation criteria

Trained assessors do not make decisions on samples (see **Figure 6**). Rather, they evaluate samples for the degree of difference from the control sample as outlined in **Section 8.4.2**. The data are then statistically analyzed to determine whether there is a significant difference (see **Section 8.5.2**).

7.3.4 Evaluation Ballots

Several types of discrimination, or difference, tests may be conducted with a panel of trained assessors (see **Section 3.3.4**), although the "difference-from-control" test has been shown to be effective in seafood tainting situations (EEM 1996). Ballots will vary according to the type of discrimination test chosen. Ballots for a difference-from-control test are illustrated in **Appendix 5**.

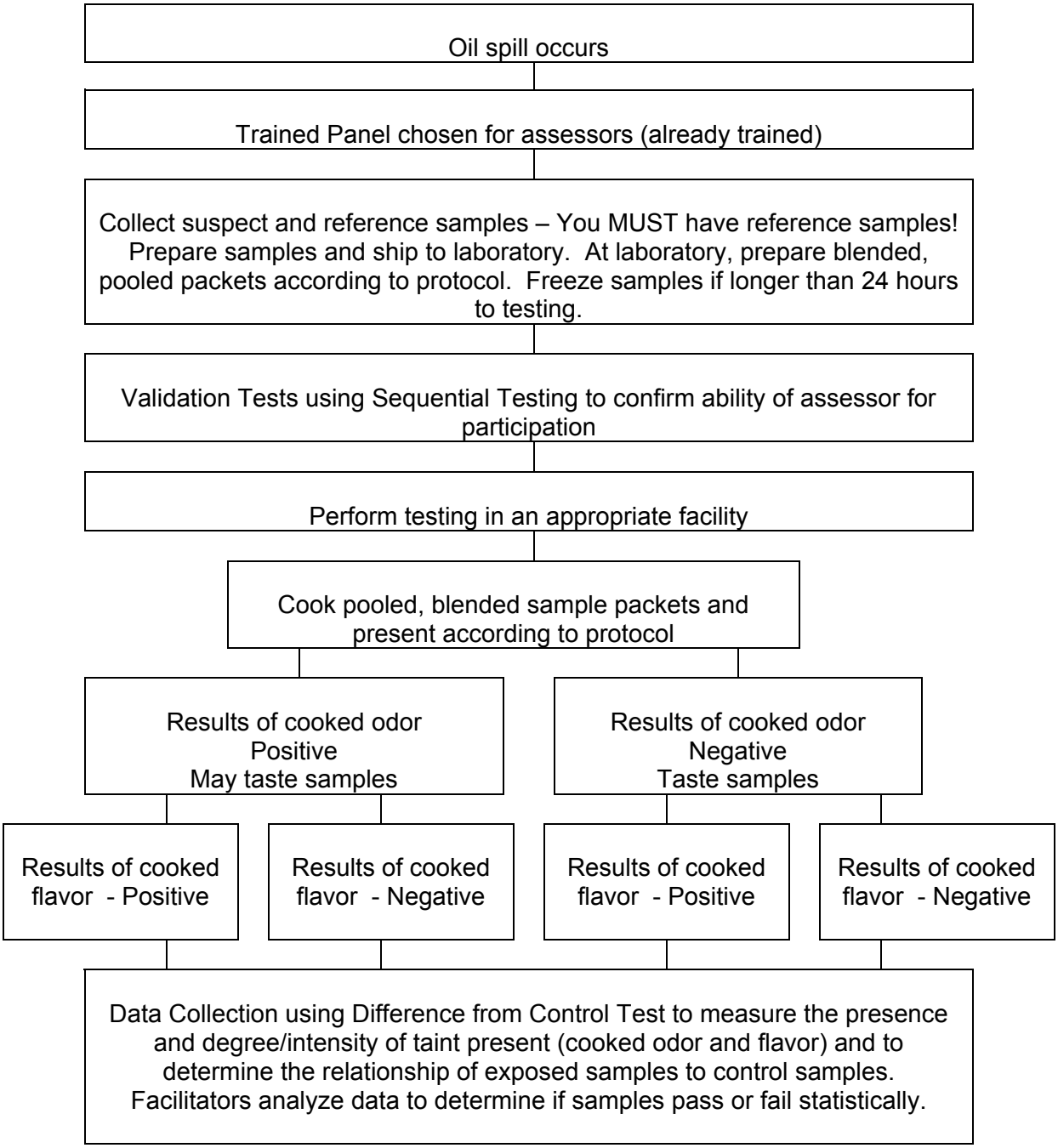


Figure 6. Sensory decision tree for use by *trained assessors* in evaluating petroleum taint in seafood.

8.0
Collection and Analysis of Test Data and Decision Criteria

8.1 Introduction

- Because seafood sensory testing for the presence of taint requires an analytically based objective response, sensory assessment must be based on this type of evaluation. As explained in **Ssection 3.2**, it is important to evaluate samples suspected of petroleum contamination ***objectively*** and not ***subjectively*** (according to likes or dislikes). If done correctly, objective sensory testing will measure the presence and intensity of a tainting substance with no allowance for like/dislike responses.
- If the decision is made to perform sensory testing after an oil spill, an overall experimental plan should be developed (see **Sections 2.0 and 3.0**), including the experimental design for collection and analysis of data. In planning the design, careful consideration must be given to the
    - type of assessors
    - type and number of samples
    - size of the experiment
    - number of replications
    - availability of reference samples
    - data collection method
    - subsequent statistical analysis
- The presence or absence of control samples is pivotal to the design and data-collection method chosen. Whenever possible, ***blind control samples*** *should* be included in the experiment to monitor the performance of expert assessors and *must* be included with trained panelists (see explanation below). Control samples must be collected immediately after the spill and handled according to procedures in **Section 2.2**. Control samples should be harvested from an unaffected area adjacent to the spill site.
- ***Each spill situation is unique!*** Although the examples given below have proven effective in petroleum-tainting situations, this document is not meant to imply that other types of data would be ineffective. We strongly recommend that

a statistician be consulted. See **Section 9.0** for sources of more in-depth information.

8.1.1 *Expert* assessors

Expert assessors, as described in **Sections 3.5 and 3.6**, are intimately familiar with "normal" sensory characteristics of seafood and any byproduct of deterioration in quality due to aging in storage, decomposition of fats, proteins, or other microbial contamination normally found in fish. Because of this familiarity, the expert assessor is able to examine samples for taint only and disregard other sensory characteristics.

- Whenever possible, control samples should be incorporated into the experimental design, but if they are not available, expert assessors can still perform the sensory testing. If control samples are not available, the expert assessor is able to detect taint comparing the samples to their internal "control" based on his past experiences and training sessions with that species (i.e., they know what "normal" is) (ISO 8586 –2).
- Generally with expert assessors, both *qualitative* (presence) and *quantitative* (intensity) information can be obtained because they have had some descriptive training. To obtain valid qualitative information (the identification of particular odor/flavor attributes), the assessor must have had some descriptive analysis training (ASTM MNL 13; ISO 8586; Rainey 1986). A suggested list of descriptors and training references relating to petroleum tainting can be found in **Appendix 2**. Seafood inspectors trained and selected for their ability to detect taint—but with no additional descriptive training—can also be used. In this case, assessors would use a pass/fail system by passing a sample if no taint is detected, or failing a sample it taint is detected.

8.1.2 *Trained* assessors

Trained assessors (see **Sections 3.5 and 3.6**) are used to conduct objective quantitative sensory testing (i.e., to test statistically for a difference between exposed and non-exposed (control) seafood). With trained panels, it is ***always*** necessary to have control samples.

- Because this type of assessor is usually not familiar with every *normal* characteristic of seafood samples, including deterioration, the difference-from-

control test will be less problematic than the other tests (see EEM document for details) which have been reported (e.g. triangle (3-AFC), paired comparison, duo-trio).

- Trained assessors are asked to rate the degree of difference from the control, and are provided a scale for this purpose.

8.2 Test data types and numbers of assessors needed

8.2.1 Data types

Data are collected to measure a sensory response, in this case, the presence of petroleum taint. Sensory data are generally considered to fall under one of the four following categories. The categories marked with an asterisk (*) are most appropriate for measuring taint.

Nominal* **Qualitative;**; in name only (i.e., yes/no, pass/fail, accept/reject). Assigning descriptors (without absolute relative intensity) also falls into this category.

Ordinal **Relative** value by ranking, but with unknown relative intervals between categories.

Interval* **Quantitative** values that increase with constant intervals.

Ratio **Quantitative** values that increase by ***orders of magnitude*** above the previous value.

8.2.2 Number of *expert* assessors needed

Because selected expert assessors are the most highly trained (see **Section 3.5**), fewer numbers of judgments on each sample are needed to draw confident conclusions on sample tainting. Because of the high rate of reliability, ***as few as 3 to 5 assessors*** can be utilized.

8.2.3 Number of *trained* assessors needed

It is recommended that ***10 to 15 panelists*** participate in the testing to ensure that conclusions can be drawn with confidence (Meilgaard et al. 1999).

8.3 Design and content of evaluation ballots

Ballots are used to record the presence or absence of taint in a sample. Ballot design is fundamental to effective and accurate sensory analysis. Ballots incorporating sensory scales and terms that solicit accurate descriptions of the samples and possible degrees of taint are essential.

An effective ballot **must***:*

1) Provide a ***standardized*** format and terminology.

2) Provide a ***meaningful permanent record***.

3) Generate data so that a difference in the score reflects a ***reproducible variation*** in the factors being scored (*intensity* of the tainting substance).

4) Reflect general agreement on ***intensity*** of the tainting substance among assessors, thus minimising the scoring range used and variability among assessors.

An effective ballot **should:**

1) Lend itself to ***wide use*** by trained assessors.

2) Lend itself to ***statistical analysis***

3) ***Generate improved assessment procedures and habits***

Examples of ballots, and assessor instructions for each type, can be found in **Appendices 3-5.**

8.3.1 Ballots for *expert* assessors

With expert assessors, ballots can incorporate from two categories (pass/fail) to more categories (e.g., none, slight, moderate, and strong) that generate information that is more detailed. In addition, if the assessors have been trained in descriptive analysis, the ballots can include a space for recording descriptors (see example in **Appendix 4**). Additional information, such as *intensity* and *characteristics* of the taint, may help decision-makers in monitoring levels and characteristics of seafood contamination and demonstrate trends in the progress of the development and cleanup of the oil spill.

8.3.2 Ballots for *trained* assessors

Panelists are asked to rate the degree of difference between a test and a control sample on a given scale (see example in **Appendix 5**). This produces data on the intensity of tainting, which is analyzed statistically for the presence of taint.

8.4 Test data analysis

This section describes the analysis of data from spill site samples and control samples included for monitoring panel performance.

8.4.1 *Expert* assessors

Expert assessors employed as seafood inspectors are accustomed to performing pass/fail tests using a product standard for comparison. The presence of any taint, no matter how slight, usually causes a sample to fail. As with any sensory test, blind control samples, if available, should be included to monitor the effectiveness of the experiment and the performance of the assessors.

Two types of statistical analyses may be applied to expert assessors:

1) Pass/Fail decisions may be analyzed using:
    a. Sampling tables for fish inspection, including the number of defective units required for the lot to fail. In this case, the tables associated with re-inspection would be used.
    b. Statistical test such as the Fishers Exact Test (see **Appendix 11**).

- Generally, data from each assessor for each sample are examined for the number of *hits* (number of samples *failed* because of positive response to taint). Because multiple assessors evaluate each sample (see **Section 6.0**), a threshold must be established by the stakeholders as to what percentage of assessors must get a *hit* for that individual sample to fail (i.e., 3/5 or 60%, 4/5 or 80%, 5/5 or 100%). A realistic situation might be that 4 to 5 positive responses for taint cause the sample to fail, 2 to 3 positive responses initiate further testing of samples from that particular area, 0 to 1 positive response causes the sample to pass. If the assessors are truly experts, there usually is much agreement and little variation among the data.

- To illustrate this, a table of data from a NOAA/FDA training workshop for selecting expert assessors can be found in **Appendix 9**. Assessors' performance can also be monitored through control samples. Data can be quickly entered into a database programmed to automatically place samples into *pass*, *fail*, or *continue testing* categories.

2) Statistical analysis of the difference from control test using analysis of variance. This allows the evaluation of the presence of differences and the calculation of the probability levels associated with these differences. Decisions can be made according to predetermined probability levels (usually $\alpha = 0.05$).

8.4.2 *Trained* assessors

The difference-from-control test is often used in quality-control situations to measure a difference. The nature of the difference is easier to define (i.e., taint) than in triangle tests, and the panelists are less influenced by other factors in the sample. The data gathered can be analyzed through standard statistical tests to give measurements of taint relative to the control samples. The test is described in detail in Meilgaard et al. (1999) and Munoz et al. (1992).

For an internal blind control, some of the test samples should be the same as the control. The resulting mean difference-from-control of the test samples should be evaluated against the difference-from-control obtained with the blind controls. This measures the *placebo effect* (Meilgaard et al. 1999).

Review and summary of the sequence of data analysis that is used for each of the steps in the test is:

1) Expert Assessors
    a) Assessor selection – using sequential testing (see **Appendix 6**) or Fisher's Exact Test (see **Appendix 11**).
    b) Sample evaluation as pass/fail decisions as well as measurements of intensity and identity of off-flavors and odors. Blind coded controls included to monitor assessor performance.

c) If data is collected on off-flavor intensity, it may be analyzed using the same procedures as for the difference-from-control test.

2) Trained Assessors
   a) Assessor selection using sequential testing as described in **Section 4.0**.
   b) Sample evaluation using the difference-from-control method and including blind coded samples to monitor panel performance.

8.5 Decision criteria

8.5.1 *Expert* assessors

After pass/fail decisions are made on each sample, the pass/fails from each harvest location are tallied. If very few samples (1 or 2) from a particular area test as *tainted*, some tainted seafood may end up in the marketplace if harvested from that area and sold. It is the responsibility of public health officials and/or seafood processors to decide what level of risk, if any, is acceptable.

8.5.2 *Trained* assessors

Generally, 25 to 50 presentations of each of the samples and the control are needed to determine the degree of difference (Meilgaard et al. 1999), i.e. 10 to 15 assessors and 3 replications of the test.

After the mean difference-from-control for each sample (and for blind controls) are calculated, results are evaluated by analysis of variance (or by paired t-test if only one sample is compared with the control). See **Bibliography** for more information on possible analytical methods.

9.0
Bibliography

Ackman, R.G, and H. Heras. 1992. Tainting by short-term exposure of Atlantic salmon to water soluble petroleum hydrocarbons. In *Proceedings of the Fifteenth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, p.757–761. Ottawa, Ontario: Minister of Supply and Services.

Ackman, R.G., E.J. Macpherson, and A.M. Timmins. 1991. The individual and combined effects of an oil dispersant and crude oil on tainting in the sea scallop. Environment Canada Technical Report. Dartmouth, Nova Scotia: Environment Canada, Conservation and Protection.

Amerine, M.A., R.M. Pangborn, and E.B. Roesler. 1965. *Principles of Sensory Evaluation of Food*. New York: Academic Press.

ASTM (American Society for Testing and Materials). 1995. Standard test method for odor in water. Rep. D 1292–86 (re-approved 1995). Philadelphia: American Society for Testing and Materials.

Aust, L.B., M.C. Gacula Jr., S.A. Beard, and R.W. Washam II. 1985. Degree or difference test method in sensory evaluation of heterogeneous product types. *Journal of Food Science* 50:511–513.

Basker, D. 1988. Assessor selection: Procedures and results. In Moskowitz H.R. (ed.), *Applied Sensory Analysis of Foods*, vol. 1, pp. 125–143. Boca Raton, Florida: CRC Press, Inc.

Bencsath, F.A., T.I. Reilly, M. DiLiberti, L.C. Hufnagle, and J.D. Barnett. 2000. Hydrocarbon analysis in petroleum tainted salmon by the use of electronic nose and dynamic headspace gas chromatography/mass spectrometry. 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach, California, p.477–478.

Bett, K.L. & Dionigi, C.P. 1997. Detecting seafood off-flavors: limitations of sensory evaluation. *Food Technology 51*(8):70–77.

Blenkinsopp, S., P. Boileau, D. Kyle, G. Sergy, and M. Fingas. 1997. How to prepare water accommodated fractions from petroleum hydrocarbons for use in aquatic toxicity testing – the basics. In *Proceedings of the Twentieth Arctic and Marine Oil spill Program (AMOP) Technical Seminar,* pp. 515–529. Ottawa, Ontario: Minister of Supply and Services.

Blenkinsopp, S. and G. Sergy. 1997. A method of exposing fish to water-accommodated fraction of oil. In *Proceedings of the Twentieth Arctic and Marine Oil spill Program (AMOP) Technical Seminar*. Ottawa, Ontario: Minister of Supply and Services. pp. 515–529.

Cain, W.S. 1970. Odor intensity after self-adaptation and cross-adaptation. *Perception & Psychophysics* 7(5):271–275.

Chambers, E. IV and E.A. Smith. 1993. Effects of testing experience on performance of trained sensory panelists. *Journal of Sensory Studies 8*(2):155–166.

Chambers, E. IV and M.B. Wolf. 1996. *Sensory Testing Methods*. ASTM MNL 26, 2d ed. Philadelphia: American Society for Testing and Materials.

Davis, H.K., E.N. Geelhoad, A.W. MacRae, and P. Howgate. 1992. Sensory analysis of trout tainted by diesel fuel in ambient water. *Water Science and Technology 25*(2):11–18.

B. de March and R.K. York, Canadian Department of Fisheries and Oceans, Canadian Food Inspection Agency, personal communication, 2000.

Doty, R.L. 1995. *The Smell Identification Test Manual*, *3rd ed*. Haddon Heights, New Jersey: Sensonics, Inc.

Doty, R.L. P. Shaman, and M. Dann. 1984. Development of the University of Pennsylvania Smell Identification Test: A standardized microencapsulated test of olfactory function. *Physiology and Behavior* 32:489–502.

Dravnieks, A. 1985. Atlas of odor character profiles. ASTM-Data Series DS 61. Philadelphia: American Society for Testing and Materials.

Dunn-Rankin, P. 1983. *Scaling Methods*. London: Lawrence Erlbaum Assoc.

Engen, T. 1982. *The Perception of Odors*. New York: Academic Press.

Environment Canada. 1997. Technical guidance document for pulp and paper environmental effects monitoring. Rep. EEM/1997/7. Ottawa, Ontario: Minister of Supply and Services.

Girardot, N.F., D.R. Peryam, and R. Shapiro. 1952. Selection of sensory testing panels. *Food Technology* 6(4):140–143.

Heil, T.P., N.A. Lane, and R.C. Lindsay. 1989. Sensory properties of thio- and alkyl-phenols causing flavor tainting in fish from the Upper Wisconsin River. *Journal of Environmental Science and Health B24*(4):361–388.

Howgate, P., P.R. Mackie, K.J. Whittle, J. Farmer, A.D. McIntyre, and A. Eleftheriou. 1977. Petroleum tainting in fish. Rapports et proces-verbaux des reunions. *Conseil permanent international pour l'exploration de la Mer 171*:143–146.

Hufnagle, L., NOAA National Marine Fisheries Service, Seattle, personal communication.

Institute of Food Technologists Sensory Evaluation Division. 1981. Guidelines for the preparation and review of papers reporting sensory evaluation data. *Food Technology 35*(4):16-17.

Institute of Food Technologists Sensory Evaluation Division. 1981. Sensory evaluation guide for testing food and beverage products. *Food Technology 35*(11):50–59.

Jellinek, G. 1985. Sensory *Evaluation of Food: Theory and Practice*. Chichester, United Kingdom: Ellis Horwood, Ltd.

Johnsen, P.B. and C.A. Kelly. 1990. A technique for the quantitative sensory evaluation of farm-raised catfish. *Journal of Sensory Studies 4*(3):189–200.

Law, R.J. and J. Hellou. 1999. Contamination of fish and shellfish following oil spill incidents. *Environmental Geosciences 6*(2):90–98.

Lawless, H.T. and G.V. Civille. 1986. The importance of language in describing perceptions. *Journal of Sensory Studies 1*(3/4):203–216.

Lawless, H.T. and G.J. Malone. 1986. The discriminative efficiency of common scaling methods. *Journal of Sensory Studies 1*(1):85–98.

Lockhart, W.L. and R.W. Danell, 1992. Field and experimental tainting of arctic freshwater fish by crude and refined petroleum products. In *Proceedings of the Fifteenth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*. Ottawa, Ontario: Minister of Supply and Services. pp. 763–771.

MacHovec, F.J. 1987. *The Expert Witness Survival Manual*. Springfield, Illinois: Charles C. Thomas.

Mearns, A.J. 1996. *Exxon Valdez* shoreline treatment and operations: Implications for response, assessment, monitoring, and research. *American Fisheries Society Symposium 18*:309–328.

Mearns, A.J. and R. Yender. 1997. Workshop on managing seafood problems during the response phase of an oil spill. In *Proceedings of the Twentieth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*. Ottawa, Ontario: Minister of Supply and Services.

Meilgaard, M.C. 1991. Current progress in sensory analysis. A review. *American Society of Brewing Chemists 49*(3):*101–109.*

Meilgaard, M.C., G.V. Civille, and B.T. Carr. 1999. *Sensory Evaluation Techniques, 3rd ed.* Boca Raton, Florida: CRC Press, Inc.

Moller, T.H., B. Dicks, and C.N. Goodman. 1989. Fisheries and mariculture affected by oil spills. In *Proceedings of the International Oil Spill Conference Proceedings*, February 13–16, San Antonio, Texas.

Motohiro, T. 1983. Tainted fish caused by petroleum compounds - A review. *Water Science Technology 15*:75–83.

Moy, L. and M. Collins. 1996. Electronic noses and artificial neural networks. *American Laboratory,* February:22–31.

Munoz, A.M., G.V. Civille, and B.T. Carr. 1992. *Sensory Evaluation in Quality Control.* New York: Van Nostrand Reinhold.

Munoz, A.M. and G.V. Civille. 1998. Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studies 13*(1):57-75.

Murray, Derek. 1992. Canada Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, personal communication.

NCASI (National Council of the Paper Industry for Air and Steam Improvement). 1987. Guidelines to methods for the analysis of the effects of effluent on the flavour of fish. *Technical Bulletin 513.* New York: NCASI.

Neilson, A.J., V.B. Ferguson, and D.A. Kendall. 1988. Profile methods: Flavor profile and profile attribute analysis. *In* Moskowitz, H.R. (ed.), *Applied Sensory Analysis of Foods*, Vol. 1. Boca Raton, Florida: CRC Press, Inc. pp. 21-41.

Nevissi, A.E., C. Martinsen, and R.E. Nakatani. 1991. Salmon tainting by untreated and chemically dispersed Prudhoe Bay crude oil. Anchorage: Cook Inlet Spill Prevention and Response and Alaska Clean Seas.

O'Mahony, M. 1986. Sensory Adaptation. *Journal of Sensory Studies 1*(3/4): 237–258.

O'Mahony, M. and N. Odbert. 1985. A comparison of sensory difference testing procedures: Sequential sensitivity analysis and aspects of taste adaptation. *Journal of Food Science 50*:1055–1058.

Persson, P.E. 1984. Uptake and release of environmentally occurring odorous compounds by fish. *Water Resources 18*(10):1263–1271.

Persson, P.E., F.B. Whitfield, and T. Motohiro. 1987. Off-flavors in the aquatic environment. *In* Persson et al. (eds.), *Proceedings of the 2nd IAWPRC International Symposium*, Kagoshima, Japan, 12-16 October. London: Pergamon Press.

Poels, C.L. M., R. Fischer, K. Fukawa, P. Howgate, B.G. Maddock, G. Persoone, R.R. Stephenson, and W.J. Bontinick. 1988. Establishment of a test guideline for the evaluation of fish tainting. *Chemosphere 17*(4):751–756.

Prichett Mangan, P.A. 1992. Performance assessment of sensory panelists. *Journal of Sensory Studies* 7(3):229–252.

Rainey, B.A. 1986. Importance of reference standards in training panelists. *Journal of Sensory Studies* 1(2):149-154.

Rainey, B.A. 1979. Selection and training of panelists for sensory testing. IFT Short Course. Chicago: Institute of Food Technologists.

Rainosek, A.P. 1996. Inherent risks in acceptance sampling. In Martin, R.E. et al. (eds), *Fish inspection, quality control and HACCP - Conference Proceedings*, May 19–24, 1996, Arlington, Virginia. Lancaster, Pennsylvania: Technomic Publishing Co., Inc.

Reilly, T.I., and R. K. York. 1994. Sensory analysis application to harmonize expert assessors of fish products. In Sylva, G., A.L. Shriver, and M.T. Morrissey (eds.), *Quality Control and Quality Assurance for Seafood*. Corvallis: Oregon State University. pp. 120–124.

Roubal, W.T., S.I. Stranahan, and D.C. Malins. 1978. The accumulation of low molecular weight aromatic hydrocarbons of Crude Oil by Coho Salmon and Starry Flounder. *Archives of Environmental Contamination and Toxicology 7*:237–244.

Rousseau, B., A. Meyer, and M. O'Mahony. 1998. Power and sensitivity of the same-different test: Comparison with triangle and duo-trio methods. *Journal of Sensory Studies 13*(2):149–173.

Rutledge, K.P. 1992. Accelerated training of sensory descriptive flavor analysis panelists. *Food Technology 46*(11):114–118.

U.S.C.F.R. (Code of Federal Regulations). 1994. Title 50, Sect. 260.57 "Sampling." Washington, D.C.: U.S. Government Printing Office.

Vie, A. and M. O'Mahony. 1989. Triangular difference testing: Refinements to sequential sensitivity analysis for predictions for individual triads. *Journal of Sensory Studies 4*(2): 87–103.

Whitford, J. 1992. Characteristics of tainting in farmed Atlantic salmon and cultured blue mussels exposed to water-soluble fractions of Brent crude oil and Scotian Shelf condensate. Dartmouth, Nova Scotia: Environmental Protection.

Whittle, K.J., D.A. Mackie, P.R. Anderson, C.F. Moffat, N.J. Shepherd, and A.H. McVicar. 1995. The impact of the *Braer* oil on caged salmon. In J.M. Davies and G. Topping (eds.), *The Impact of an oil spill in turbulent waters: The Braer. Proceedings of a symposium held at the Royal Society of Edinburgh*, 7-8 September, p.121-143 and 144-160. Edinburgh: The Stationery Office, Limited. ISBN 0 11495798 3.

Whittle, K.J., J. Murray, P.R. Mackie, R. Hardy, and J. Farmer. 1977. Fate of hydrocarbons in fish. Rapports et proces-verbaux des reunions. *Conseil permanent international pour l'exploration de la Mer,* 171:139–142.

Williams, U.P., J.W. Kiceniuk, J.E. Ryder, and J.R. Botta. 1988. Effects of an oil spill on American lobster *(Homarus americanus)* from Placentia Bay, Newfoundland. *Canadian Technical Report of Fisheries and Aquatic Sciences 1650*.

York, R.K., and L.M. Sereda. 1995. Sensory assessment of quality in fish and seafood (Chap. 13). *In* Shahidi, F. and J.R. Botta (eds.), *Seafoods: Chemistry, Processing Technology and Quality.* London: Blackie Academic and Professional. pp. 233–262.

York, R.K. 1989. Canadian fish products-fish inspection and sensory evaluation. Canadian Institute of Food Science Technology Journal *22*(5): 441–444.

York, R.K. 1995. Quality assessment in a regulatory environment. *Food Quality and Preference 6*:137–141.

Zelek E.F. Jr. 1990. Legal aspects of sensory analysis. *Food Technology 44*(11):168-174.

Zook, K.L., and J.H. Pearce. 1988. Quantitative descriptive analysis. *In* Moskowitz, H.R. (ed.), *Applied Sensory Analysis of Foods*, vol. 1. Boca Raton, Florida: CRC Press, Inc. pp. 43–71.

Zook, K., and C. Wessman. 1977. The selection and use of judges for descriptive panels. *Food Technology 31*(11): 56–61.

APPENDIX 1

Normative References - Existing Guidelines, Standard Practices, And Sampling Plans for Sensory Testing

American Society of Testing & Materials (ASTM) Standards

Standard practice for evaluating an effluent for flavor impairment to fish flesh. ASTM D3696-89.

Standard practices for evaluating effects of contaminants on odor and taste of exposed fish. Committee E-18 on Sensory Evaluation of Materials and Products. ASTM E1810-96. [*Supersedes* the sensory evaluation procedures in D3696-95, Standard practices for evaluating an effluent for flavor impairment of fish flesh, in *Standardization News*, Jan. 1997, p. 4.]

Terminology relating to sensory evaluation of materials and products. ASTM E253-98a.

Standard practices for referencing supra-threshold odor intensity [re-approved 1993]. ASTM E544-75.

Standard test method for sensory analysis – triangle test. ASTM E1885-97.

Manual on descriptive analysis testing for sensory evaluation. ASTM MNL 13. 1992.

Manual on sensory testing methods. ASTM STP 434. 1999.

Establishing conditions for sensory evaluation of foods and beverages. ASTM STP 480-84.

Guidelines for the selection and training of sensory panel members. ASTM STP 758. 1981.

Physical requirement guidelines for sensory evaluation laboratories. ASTM STP 913.

Canadian Government Standards

Technical guidance document for pulp and paper environment effects monitoring. Environment Canada EEM/1997/7 October 1997.

International Organization for Standardization (ISO)

Sensory analysis – General guidance for the selection, training and monitoring of assessors – Part I: *Selected assessors*. ISO 8586-1. 1993.

Sensory analysis – General guidance for the selection, training and monitoring of assessors – Part 2: *Experts*. ISO 8586-2. 1993.

Sensory analysis – Vocabulary. ISO 5492. 1992.

Sensory analysis – Methodology – General guidance. ISO 6658. 1985.

Sensory analysis – General guidance for the design of test rooms. ISO 8589. 1998.

Sensory analysis – Methodology – Triangular test**.** ISO 4120. 1983

United Nations/World Health Organization (UN/WHO)

UN/WHO Codex Alimentarius Commission. Draft guideline for the sensory evaluation of fish and shellfish in laboratories. ALINORM 99/18.

UN/WHO Codex Alimentarius Commission. Procedural Manual F/3026.

Sampling Plans for Sensory Testing of Seafood

American National Standards Institute (ANSI)/American Society for Quality Control (ASQC). Sampling procedures and tables for inspection by attributes. ANSI/ASQC Z1.4. 1993.

International Organization for Standardization (ISO).

Sampling procedures for inspection by attributes. ISO 2859.

Part 1: Sampling plans indexed by *acceptance quality level (AQL)* for *lot-by-lot* inspection.

Part 2: Sampling plans indexed by *limiting quality (LQ)* for *isolated lot* inspection.

UN/WHO Codex Alimentarius Commission. Procedural Manual F/3026.

U.S. Code of Federal Regulations. Title 50, Sect. 260.57, "Sampling." 1994 (example of an AQL sampling plan).

APPENDIX 2

Definitions, Terminology, and References used in Sensory Training for Petroleum Taint

Specific Seafood Terminology

Associated with *fresh seafood*, not subject to deterioration

Odor: Ocean air, clean seaweed, briny, slightly sweet, metallic, cucumber, melon, grassy, neutral.

Flavor: Slightly sweet, oceany, meaty/brothy, briny, fresh oil, buttery, neutral.

Associated with *seafood deterioration* (spoilage)

Odor: Fishy, oxidized, rancid, sour, fermented, yeasty, fruity, sulfury vegetable, pungent, putrid, fecal, cheesy, ammonia, painty.

Flavor: Fishy, oxidized, rancid, painty, sour, bitter, yeasty, fermented, sulfury vegetable, putrid, fecal.

Associated with *petrochemical tainting* of seafood

Odor: Petroleum, diesel, kerosene, hydraulic fluid, lube oil, dirty sour (old oil), solventy, chemical, pungent, acrid, burnt, creosote, tar, piney/resinous, rubbery, phenolic, nose-sting.

Flavor: Pungent, kerosene, petroleum fuel, chemical, dirty sour, warming mouth-feel, oily/waxy mouth-coating.

General Sensory Definitions

acrid/burnt	Burning, irritating, pungent, aromatic; often associated with burnt wood or smoke.
adaptation	Decreased in sensitivity to a given stimulus resulting from exposure to that stimulus.
ammonia	Aromatic characteristic of unscented ammonia.
anosmic	Lack of sensitivity to odor stimuli.
appearance	All the visible characteristics of a substance/sample.
assessor	Any person taking part in a sensory test.

astringent	The chemical "feeling" factor on the tongue or other skin surfaces of the oral cavity, described as puckering/dry and associated with tannins or alum.
bakelite	Burnt, phenolic, aromatic; reminiscent of a burnt circuit board or burnt plastic.
bitter	One of the four basic tastes (w/sour, sweet, salty) primarily perceived at the back of the tongue; common to caffeine and quinine.
briny	Aromatic associated with the smell of clean seaweed and ocean air.
brothy	Aromatic associated with boiled meat.
burnt	Aromatic associated with heated, scorched, or blackened substances.
burnt plastic	Aromatic associated with burnt plastic, such as Bakelite.
burnt rubber	Aromatic associated with higher sulfur mercaptans.
cardboardy	Aromatic associated with slightly oxidized fats, reminiscent of wet cardboard packaging.
carry-over	A decrease in sensitivity to a given stimulus resulting from exposure to previous samples containing the same stimulus.
cheesy	Sour aromatic associated with aged cheese and butyric acid.
chemical	A general term associated with many types of aromatic compounds such as solvents, cleaning compounds, and hydrocarbons.
creosote	Heavy, tar-like, acrid aromatic associated with creosote, smoke, and some solvents.
cucumber	Aromatic associated with fresh cucumber; similar aromas can be associated with certain species of very fresh, raw fish.
decompose	Break down into component parts; fish having an offensive or objectionable odor, flavor, color, texture, or substance associated with spoilage.
diesel	Chemical-like aromatic associated with diesel fuel.
dirty	Soiled, sour aromatic.
distinct	Capable of being readily perceived.
earthy	Aromatic characteristic of wet foliage, damp soil, or slightly undercooked boiled potato.
error	Difference between the observed value and the true value.
error/psychological	Errors that can introduce bias into sensory assessment.
error/physiological	Systemic errors that may be positive or negative. These differ from the statistical phenomena of random error, which are unpredictable errors that are random deviations of observed values from true values and that average to 0.

fatigue	See Adaptation.
fecal	Unpleasant aromatic associated with complex protein decomposition.
feel	A chemical sensation in the nose or mouth, such as astringent, cooling, pungent.
fermented	Sour aromatic associated with rotting fruit or vegetables.
fishy	Aromatic associated with old, lower-quality fish, as demonstrated by the odor of tri-methylamine (TMA) or cod liver oil.
flavor	Perceived attributes of a food substance when placed in the mouth resulting from the stimulation of taste, odor, and feeling factors.
freshness	Concept relating to time, process, or characteristics of seafood as defined by a buyer, processor, user, or regulatory agency.
fruity	Aromatic associated with slightly fermented fruit. In seafood, fruity odors generally result from high-temperature spoilage.
fuel oil	General term to describe the aromatics of fuel oils such as diesel or kerosene.
gasoline	Aromatic associated with gasoline.
grassy	Green, slightly-sweet aromatic associated with cut grass or very fresh, high-quality finfish.
hydrocarbon	Aromatic associated with fuel-combustion products.
intensity	Perceived magnitude of a sensation.
iridescent	Array of rainbow-like colors, similar to an opal or oil sheen on water.
kerosene	Aromatic associated with kerosene.
lube oil	Heavy, greasy aromatic associated with lube/motor oil.
masking	Phenomenon in which one sensory attribute obscures or diminishes one or several other attributes present.
mercaptan	Aromatic associated with sulfur compounds, reminiscent of skunk and rubber.
metallic	Aroma and/or taste associated with ferrous sulfate or tin cans.
moldy	Aromatic-associated mold growth; for example, an old, damp basement.
mouth-coating	Sensation of a film coating the inside of the mouth.
mouth-filling	Sensation of fullness dispersing throughout the mouth.
musty	Aromatic associated with a moldy, dank cellar.
noseburn	Chemical "feeling" factor described as a warmth or burning sensation in the nasal passages when a product is sniffed.
odor	Sensation caused by stimulation of the olfactory receptors in the nasal cavity by volatile material.

off-odor	General, non-specific term relating to characteristics that are inappropriate in a food system, usually related to aging or contamination.
oxidized	Aromatic associated with rancid, stale, painty, or cardboardy.
persistent	Existing without significant change; not fleeting.
petroleum	Aromatic associated with any material of petrochemical hydrocarbon nature.
phenolic	Harsh, irritating, medicinal aromatic associated with phenol or plastic Band-Aids.
pine-like	Resinous pine-tree type aromatic; may be slightly medicinal or disinfectant-like.
pungent	Irritating, sharp, or piercing sensation.
putrid	Aroma associated with decayed meat, usually resulting from anaerobic decomposition.
quality	Degree of excellence. A collection of product characteristics that confers its ability to satisfy stated or implied needs.
rancid	Odor or flavor associated with rancid oil. Gives a mouth-coating sensation and/or a bitterness perceived on the back of the tongue. Sometimes described as painty.
reference	Either a sample designated as the one to which others are compared, or another type of material used to illustrate a characteristic or attribute.
resinous	Medicinal, woody aromatic.
rubbery	Sulfurous, phenolic aromatic associated with rubber products.
salty	Taste on the tongue associated with salt, sodium chloride, or odor of brine.
sensory	Relating to the use of the sense organs.
solventy	Odor and/or nose "feel" or flavor associated with solvents such as acetone.
sour	Odor and/or taste sensation, generally due to the presence of organic acids.
stale	Odor or flavor associated with wet cardboard or frozen storage.
sulfury	Odor or flavor associated with sulfur-based materials such as matches, old garlic, onions, rotten eggs, mercaptans, or rubber.
sweet	One of the four basic tastes on the tongue(w/bitter, sour, salty) stimulated by sugars. Also a sweet odor, such as vanilla extract.

tar-like	Aromatic associated with the heavier, tar-like substances of petroleum chemicals, as demonstrated by hot asphalt.
taste	One of the basic senses, the receptors for which are located in the mouth and activated by compounds in solution.
terminology	Terms used to describe the sensory attributes of a product.
umami	Taste produced by substances such as monosodium glutamate (MSG) in solution. A meaty, savory, or mouth-filling sensation.
vegetable	Odor associated with sulfur-containing vegetables such as cooked broccoli, cabbage, or cauliflower.
watermelon	Aroma characteristic of fresh-cut watermelon rind. Similar odors sometimes found in certain species of very fresh, raw fish.
yeasty	Aroma associated with yeast and fermented products such as bread or beer.

Petroleum Taint References for Assessor Training

As explained in more detail in **Section 4.0**, it is very important to include actual petroleum-contaminated seafood samples when selecting, testing, and training sensory assessors, whether expert or novice. Actual samples can serve as both controls and references (see **Section 4.0**). In addition, the substances listed below can serve as referents for developing and refining sensory terminology during the assessor training process.

Term	Referent
astringent (oral)	alum solution
burnt/acrid	burnt toast, scorched oil
burnt plastic	burnt plastic
cooling (nasal)	menthol, etc.
creosote	creosote
diesel	diesel fuel
earthy	beets, potatoes, potting soil
gasoline/kerosene	gasoline/kerosene
hydraulic fluid	hydraulic fluid
lube oil	WD-40
petroleum	petroleum jelly
phenolic	phenol, Band-Aids
piney	pine needles, Pine Sol
pungent (nasal)	vinegar, burnt substances
rubber	rubber bands
soapy	lauric acid
solventy	acetone
sour	old oil, vinegar, yogurt, etc.
sulfur	rubber, matches, old garlic/onions, mercaptans
tar-like	tar

APPENDIX 3

Expert Assessor Ballot for Recording Sensory Evaluations of Seafood: 3-Alternative Forced-Choice (AFC) Triangle Test

Name _____

Date _____

Product _____

Sensory panel screening

Six sets of three samples each are laid out in the room. Two of the three samples smell the same.

Using this ballot, evaluate each sample set and identify the *one sample that differs* from the other two.

Open and evaluate the samples as shown, taking two or three shallow sniffs. Evaluate the odor in each sample for the presence of taint.

Within each set, evaluate the samples in the order given below. You may evaluate a sample more than once, if needed. Partially close the sample, as shown, when not testing it.

Open each sample only briefly to prevent any release of odor into the test room. If you cannot detect any difference among the samples, you must guess.

	Sample sets			
	Sample #	**Sample #**	**Sample #**	**The one sample that is different.**
1	435	394	391	
2	433	450	717	
3	183	462	245	
4	843	700	906	
5	591	845	056	
6	130	801	295	

Comments:

APPENDIX 4

Expert Assessor Ballot for Recording Quantitative/Qualitative Sensory Evaluation of Seafood.

Name:_____

Date:_____

Species: _____

Open and evaluate the samples as shown. Evaluate each sample for presence of "off" odor or flavor.

Make a Pass/Fail decision, rate the intensity (using the numerical scale), and characterize (using descriptors).

RO = Raw odor
CO = Cooked odor
CF = Cooked flavor

		Decision		**Intensity scale**				**Comments/Descriptors**
		Pass	**Fail**	**None**			**Strong**	
051	RO			0	1	2	3	
051	CO			0	1	2	3	
051	CF			0	1	2	3	
		Pass	**Fail**	**None**			**Strong**	
333	RO			0	1	2	3	
333	CO			0	1	2	3	
333	CF			0	1	2	3	
		Pass	**Fail**	**None**			**Strong**	
758	RO			0	1	2	3	
758	CO			0	1	2	3	
758	CF			0	1	2	3	
		Pass	**Fail**	**None**			**Strong**	
420	RO			0	1	2	3	
420	CO			0	1	2	3	
420	CF			0	1	2	3	

APPENDIX 5
Trained Assessor Ballot for Recording Sensory Difference-from-Control Evaluations of Seafood.

Name: _____ Date: _____

Species: _____ Test or Set #: _____

Attribute assessed = Presence of **Taint** in **Cooked Odor** or **Cooked Flavor**

Do not swallow samples: **EXPECTORATE** any sample that is tasted.
Follow strict rinsing procedures.

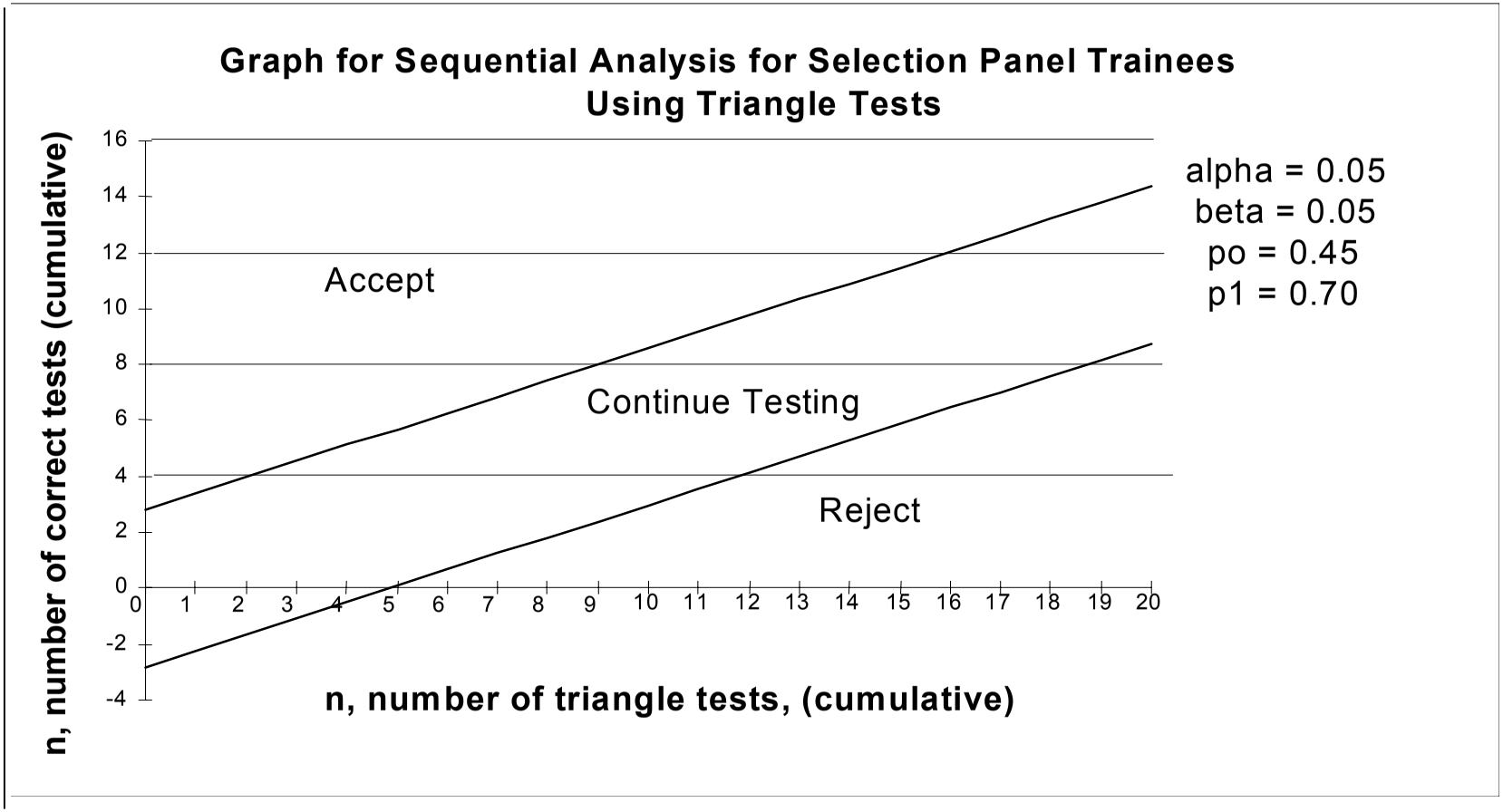
Instructions:

1. Smell and taste the sample marked "Control" first.
2. Smell the sample marked with the 3-digit code; if no taint is detected, or if it so slight as to be questionable, taste the sample.
3. Assess the overall sensory difference between the two samples, using the scale below.
4. Mark the scale to indicate the degree of overall difference by placing a vertical mark across the horizontal line at the point corresponding to the degree to which the sample deviates from the reference (indicated by **R**).

Code No.	Sensory Scale	
_____	*same as* R	*extremely different* from R
_____	*same as* R	*extremely different* from R
_____	*same as* R	*extremely different* from R
_____	*same as* R	*extremely different* from R
_____	*same as* R	*extremely different* from R
_____	*same as* R	*extremely different* from R

APPENDIX 6

Control graph for sequential analysis using the triangle test method for assessor selection.



APPENDIX 7

Method for Exposing Live Seafood to Petroleum Products for Selecting and Training Sensory Assessors.

Memorandum Report (February 2000)

Larry Hufnagle
Environmental Conservation Division
Northwest Fisheries Science Center
National Marine Fisheries Service, NOAA
Seattle, Washington 98112

Three commercially important seafood species were exposed to 25-ppm diesel or Monterey Crude oil to produce sensory-analysis training materials for the National Marine Fisheries Service's (NMFS) Sensory Branch Inspection Services and the U.S. Food and Drug Administration. The exposure was conducted at the Mukilteo Field Station of the Northwest Fisheries Science Center in Mukilteo, Washington. Three classes of experimental organisms were chosen for the experiment—a bivalve (oyster), crustacean (lobster), and finfish (Atlantic salmon)—representing commercially important seafood types. Oysters (*Crassostrea gigas*) size #1 were obtained from Westcott Bay Sea Farms, Friday Harbor, Washington. Two sizes of Atlantic salmon (*Salmo salar*)—2 lb. fish for the diesel exposure and 1 lb. fish for the Monterey Crude oil exposure—were obtained from Northwest Seafarms, Bainbridge Island, Washington. The lobsters (*Homarus americanus*), weighing 1 to 1.25 lbs. each, were shipped from Gloucester, Massachusetts.

- The *diesel exposure tank* contained 46 salmon, 40 lobsters, and 240 oysters (assuming 6 oysters/sample) to obtain the required 25 to 30 samples per species. The *control* tank contained 25 salmon, 20 lobsters and 120 oysters.

- The *Monterey Crude exposure tank* contained 40 salmon, 40 lobsters, and 240 oysters. The *control* tank contained of 35 salmon, 20 lobsters and 120 oysters.

The surplus specimens were to allow for incidental mortality during transportation and exposure, and for monitoring of baseline contamination and uptake during the exposure.

The control tanks were used to rule out any flavors or odors that might be artifacts of our facility (e.g., from the water or the tanks) and to serve as controls for chemical analysis. The *control* organisms (3 species) were held in an 8-ft circular tank (4981.6 L), and the *exposed* organisms (3 species) were held in a 12-ft circular tank (10129.2 L). A PVC liner was used to prevent oil contamination of the aquaculture facilities' tanks. All three species were allowed to acclimate for at least 24 hours in their respective tanks (under flow-through conditions) before exposure. Exposure consisted of 48-hour static-water exposure with aeration and a single addition of the petroleum product to yield a nominal concentration of 25-ppm (w/v). Approximately 250 g of the diesel or Monterey Crude oil were weighed out in a beaker. At the beginning of the exposure, water flow and aeration were turned off and the petroleum product was poured onto the water surface. For diesel oil, the beaker was rinsed several times in the tank water to complete the transfer, and the aeration was then resumed. For the crude-oil exposure, the oil had to be scraped out of the beaker to complete the transfer. The aeration system was used to: 1) circulate the petroleum product in the tank,
2) simulate water conditions characteristic of near shore exposure, where wave action and mixing are an important part of the exposure conditions, as well as
3) maintain the system-dissolved oxygen during the static exposure.

At the end of the 48-hour exposure, the animals were sacrificed. Salmon were filleted, skinned, wrapped in aluminum foil, and vacuum-packed before storing at -20° C. Whole lobsters and oysters in the shell were wrapped in aluminum foil and vacuum-packed before storing at -20° C.

APPENDIX 8

Using Random Code Numbers and Random Sample-Presentation Methods

Random code numbers are those code numbers that cannot be predicted, based on the numbers already seen in the sample series, and that bear no relationship to any variable within the samples.

An example is shown in a series that can be predicted and one that cannot, where a coin is tossed and, assuming that 0 = heads and 1 = tails, these series become:

predictable series. . . coin 0 1 0 1 0 1 0 1 0 1 0 1. . . . . .

unpredictable series. . . coin 1 1 0 0 0 1 0 1 0 1 1 1 0 1 1 0 0 1 0 0 0 0 1. . . . . .

Random numbers are used in sample presentations to 1) prevent assessors from predicting information about the samples, and 2) control the occurrence of errors of expectation.

Information about samples can be inadvertently given from factors such as

- the order in which samples are presented,
- the code numbers assigned to the samples, or
- extraneous information about the samples that have been given before the evaluation sessions.

Also, seemingly predictable codes can provide false information that creates confusion in a test. These errors are generally not an issue when the evaluators are professional sensory analysts who have been calibrated and tested. However, these errors are significant when selecting assessor trainees and testing trained sensory panels.

These presentations are appropriate for both expert and trained assessors to demonstrate the validity of the test method.

The randomization process

1. Assigning code numbers to the sample. In this case, 3-digit random number codes are used. This is common practice in sensory testing, as 3-digit codes generally have no meaning to the assessors. (However, there may be occasions where 3-digit codes do have meaning to the assessors in the context of the samples. In this case, using 4-digit codes will

generally remove the difficulty.)

2. Assigning random presentation sequences for the samples. This reduces any effect of sample order or carryover effect from one sample to another. Each assessor examines the samples in a different sequence from the others. This is done when testing both expert and trained assessors.

Generating 3-digit code numbers

- Examples of **predictable** coding that should ***not*** be used. Using a meaningful code within the sample number, e.g., the middle number:

113	219	817	blank (water)
321	726	429	low-concentration salt
138	432	635	high-concentration salt

- Examples of **unpredictable** coding as it should be used. An appropriately coded series would be given by no meaningful numbers:

726	255	979	blank (water)
368	417	182	low-concentration salt
631	118	229	high-concentration salt

- **Random 3-digit number codes** are easily generated in different ways:

  1. Using the **random number table**, found in the tables section of most sensory analysis texts and statistics texts. A starting point within the table is arbitrarily selected, and random codes are written from that point. Resulting codes should be checked so that no accidental patterning occurs and so that no repeat codes are generated.

  2. Using a **random code set**, i.e., a prepared set of all the 3-digit codes from 001 to 999 on separate cardboard disks (or other convenient medium). The codes are drawn from this set without replacement, to ensure that no repeat codes are generated. The resulting number set should be checked for any accidental patterning.

1. By computerized random number generation within a software package, e.g., Excel. This is the easiest way to generate a large set of random numbers; the sort function can be used to check for any repeat codes.

Generating random 3-digit numbers using Excel™.

This method will generate a set of 3-digit random codes with no repeats for use in coding samples for sensory studies. The codes act to remove identity from the sample for presentation to sensory panelists, but still provide a method for tracking samples within a study.

A. Generating the random number set:

1. Using "TOOLS" > "DATA ANALYSIS" > "RANDOM NUMBER GENERATION", generate the set of three digit codes needed.

   Settings:

   No. of variables = 1.

   No. of random variables = (as needed).

   Distribution = uniform.

   Between 1 and 999 (or 100 and 999 if numbers below 100 are not needed).

   Random seed = choose one as needed.

   Output range = as needed (Column A, here).

2. Format the code numbers using "FORMAT" "CELL" "NUMBER" "CUSTOM" to 000 (so that 1 to 99 will appear as 3 digits).
3. Adjust the values of the numbers generated in the worksheet using "TOOLS" > "OPTIONS" > "CALCULATION" and check "precision as displayed", and "OK" to loosing the accuracy with the numbers. (This gets rid of hidden decimal places that interfere with the sorting process).

B. ***Now begin the process of checking for duplicates by:***

4. Enter "1" in the first cell of Column B. Number the codes from 1 to X (here = 100) in column B (so that they can be put beck in random sequence after checking duplicates). Do this with "EDIT" > "FILL" > "SERIES" > "COLUMNS" (step value = 1, stop value = N, (hers, 100)).
5. Use "DATA" > "SORT" by Column A to put the random numbers in order.
6. Place the cursor in the first cell of Column C. Using the "Function Wizard" icon, call up the IF statement, and enter the formula to test for repeat numbers into Column C, with "a1 = a2, if true = 1, if false = 0". Copy this statement down the full length of Column C.

7. Check Column C for any 1's. Correct and duplications (i.e. change them appropriately). (By entering a "Σ" command at the base of Column C, the number of duplicates which must be corrected will be shown. As they are corrected, this number will reduce to "0").
8. Use "DATA" > "SORT" by Column B to put the codes back in random order. Assign them to samples as needed.

APPENDIX 9

Example of *expert assessor* training data
(from NOAA Seafood Safety Workshop, May 1999, Seattle, WA).

Summary scores following brief (8-hr) training period.

Sample Key
C = Control (no exposure)
D = Diesel fuel exposure
M = Monterey Crude oil exposure

Score Intensity Key
0 = no contamination
1 = very slight
2 = slight contamination
3 = moderate contamination
4 = strong contamination

Salmon				#	Oysters				#	Lobster				#	Overall %	Assessor
C	D	C	M	Correct	C	M	D	C	Correct	D	C	M	D	Correct	Correct	#
0	2	0	3	4	0	3	3	0	4	2	0	2	3	4	100.00	5
0	1	0	3	4	0	2	2	0	4	3	0	3	2	4	100.00	7
0	2	0	2	4	0	1	2	0	4	2	0	1	2	4	100.00	8
0	2	0	1	4	0	1	3	0	4	3	0	1	3	4	100.00	11
0	1	0	1	4	0	1	2	0	4	2	0	2	3	4	100.00	12
0	2	0	3	4	0	3	2	0	4	3	0	2	3	4	100.00	13
0	1	0	2	4	0	1	2	0	4	2	0	2	2	4	100.00	15
0	2	0	2	4	0	1	2	0	4	2	0	2	2	4	100.00	16
0	2	0	3	4	1	1	1	0	3	2	0	2	3	4	91.67	1
0	2	0	2	4	0	2	3	0	4	3	0	0	3	3	91.67	3
0	1	0	2	4	0	2	2	0	4	2	0	0	3	3	91.67	6
0	2	0	1	4	0	1	3	0	4	3	0	0	1	3	91.67	10
0	1	0	3	4	0	1	1	0	4	3	0	0	3	3	91.67	14
1	0	0	2	2	0	2	2	0	4	3	0	1	2	4	83.33	4
1	1	0	2	3	0	1	2	0	4	1	0	0	1	3	83.33	17
0	3	0	2	4	1	0	2	0	2	2	0	0	1	3	75.00	2
1	0	0	2	2	0	3	3	0	4	3	0	1	0	3	75.00	9
0	1	0	2	3.79	0	2	2	0	3.85	2	0	1	2	3.584	**92.65**	**Group Avg.**
3	15	0	17		2	17	17	0		17	0	11	16			

Average petroleum intensity
Total hits in 17 evaluations

These data are from a training workshop using known samples. Participants were seafood inspectors or seafood researchers. For an actual oil-spill situation, you would select those assessors scoring 100%, if possible, or at least 90%. When using assessors for seafood testing who score less than 100%, you may want to increase the number of assessors.

APPENDIX 10

NOAA/NMFS NATIONAL SENSORY SECTION
CHAIN OF CUSTODY FORM

7600 Sand Point Way NE, Seattle, WA
For more information contact Michael DiLiberti
978-281-9123 or FAX 978-281-9125

Project_____ **Sampler**_____

Sample I.D. #	Date Collected	Location	Sample Type (Tissue, oil, water. Include species name and tissue type)	Comments
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□	□	□
□	□	□		
□	□	□	□	□

Collected by: *(signature)*□	**Received by:***(signature)*□	**Condition:**□	**Date/Time**□
Relinquished by: *(signature)*□	**Received by:***(signature)*□	**Condition:**□	**Date/Time**□

Relinquished by: *(signature)*	**Received by:***(signature)*	**Condition:**	**Date/Time**

* If shipped, include carrier name and copy of shipping invoice

Chain of Custody Procedures

BACKGROUND

The NOAA Damage Assessment Center (DAC) has established chain of custody procedures for those personnel collecting field data. This document is a record of the methods used to handle samples collected for the damage assessment process, which may become evidence in a court of law.

The procedures outlined in this document represent one acceptable method. The failure in any particular instance to follow one or more of the steps listed here does not necessarily render evidence either inadmissible or unusable, however, field personnel should inform either the Damage Assessment Center or its attorneys about any deviation from procedures specified in this document. All that the Courts require is that the sample itself (or results of its analysis) be adequately authenticated to assure that what occurs in court replicates circumstances existing at the sample-taking location. No defendant or respondent may claim as a defense or an objection any deviation from procedures described in this memo.

DEFINITION OF CUSTODY

"Chain-of-custody" procedures are followed to "authenticate" a sample from the time it is taken until the results are introduced as evidence into court. A sample is in your "custody" when:

1. It is in your actual physical control and presence.

2. It is in your view.

3. It is not in your physical presence but is secure in a place of storage to which only you have access.

4. It is not in your view or physical presence but is secured in a place of storage to which only you and identified others have access.

SAMPLE COLLECTION

1. As few people as possible should handle the sample from its taking through laboratory analysis.

2. Preprinted DAC samples tags should be used to identify each sample. These are filled out in waterproof ink and attached to the sample container at the time the complete sample is collected. The preprinted tags contain the following information: sample #, contents, preservative, time/date of collection, location, and collector. A witness who has observed the samples being taken should also include his/her name on the sample. The sample tags are records made in the usual course of regularly conducted official activity and may constitute past recollection recorded. When tags are completed, the collector should not leave any blanks thereon unfilled.

3. Blank samples using water prepared specifically for the sample collector should be used on a sampling project, and should be analyzed later to establish the lack of contamination by the sampling device, the container, or any preservatives used.

4. Field Data Record logbooks with numbered pages should be used to record field measurements and other pertinent information. These notes will be used to refresh the sample collector's memory in the event he/she later testifies regarding his/her actions. The original or first impression copy of these field data sheets, chain-of-custody sheets, and analysis requested sheets should be sent along with the samples. Data entered in the logbooks or forms are recorded with ballpoint pen or waterproof ink. Each page is signed by the sample collector and any available witness. The preparation and custody of the logbooks during the survey are the responsibility of the survey coordinator. Once the survey is complete, field data logbooks and data forms are to be retained by the survey coordinator or a designated representative. Any errata in entries should be lined out with a single line and initialed and dated so a later reader can read what was written before the correction.

5. The sample collector is responsible for the care and custody of the samples until they are properly dispatched to the receiving laboratory or turned over to an assigned custodian or courier. The sample collector must assure that each sample is in his/her "custody" so no one can tamper with it.

6. If colored slides, photographs, or other related evidence are obtained to show the impact of the pollutant or substantiate any other conclusions of the investigations, the following documentation should be on the back of the photo or in the field data logbook: time, date, site location, and the signature of the photographer and any witness. Film or other materials of this nature which may be used as evidence, may also (but need not) be handled using chain-of-custody procedures.

TRANSFER OF CUSTODY AND SHIPMENT

1. Collected samples are to be accompanied by a chain-of-custody record that includes the name of the survey, sample collector's initials, laboratory sample number, and number of containers. When turning over the possession of a part or all of the samples to a field analysis station or to a laboratory, the transferor and transferee should sign and record the time and date on the sheet.

2. All packages are to be accompanied by the chain-of-custody record identifying the contents. The original accompanies the shipment, and a copy is retained by the survey coordinator. The chain-of-custody record is signed by the collector along with recording the date and time. It is then placed inside the shipping container along with the Field Data Sheet and Analysis Requested Sheet.

3. Samples are to be packed and sealed for shipment in suitable containers to avoid damage. A sample seal should be attached across the lid of each shipping container in such a manner that the container cannot be opened without breaking the seal. This lock and/or seal is not to be removed until the shipping container is opened by the laboratory custodian or a designee.

4. If sent by mail, the package is sent via Registered Mail with Return Receipt Requested. If sent by common carrier, all shipping receipts should be retained as part of he permanent chain-of-custody documentation.

5. Couriers picking up samples at the airport, post office, etc. should sign the shipping documents to acknowledge receipt of the samples.

LABORATORY CUSTODY PROCEDURES

The following procedures should be followed regarding the handling and custody of samples in the laboratory. In any given instance, an assistant may perform any item indicated below, but a record should be made of the assistant's participation.

1. A designated custodian should:

    a. Accept and receive custody of the shipped samples.

    b. Observe the physical condition of the shipping container noting any broken seals or indications of any tampering.

    c. Open the shipping container (and at his/her option saving or not saving any seals or stickers on that container).

    d. Crosscheck and verify the information on the chain-of-custody receipt with the tags or other markings on the sample containers.

    e. Make appropriate entries in the chain-of-custody receipt making sure the identity of the courier is reflected.

    f. Enter or have an assistant enter the information contained on the sample tags and/or markings on the field data sheet and on the analysis-requested sheet, into a bound logbook.

    g. Place all of the received samples in an appropriate storage area that is capable of being secured against access whether or not the access to the building is secured.

2. A designated person should routinely be in charge of distributing samples to the appropriate analysts, or each analyst should be in charge of collecting his/her samples from the storage area. In any event, the person performing that activity should be identified in the analysis records for each sample.

3. When not needed or being used in the laboratory for analysis, the unused portion of the sample should be returned to its storage area.

4. All identifying tags, seals, or stickers from the sample container should be retained with the analysts notes and other analysts documents.

5. Each analyst is responsible for the care and custody of each sample received by him/her for analysis form the time of receipt until the sample is exhausted or returned to its storage area. Samples should be returned to a storage area by

the analyst who analyzed the sample, and the analysts' records for the sample should record any deviation from that practice.

6. The laboratory director should obtain authorization from the project coordinator and case attorney for sample disposal for civil cases. No criminal cases samples are to be disposed of until the case is closed and all appeals have been heard. The prosecuting attorney must be consulted prior to disposal of any evidence for criminal cases.

LABORATORY DOCUMENTATION

The procedures generally recognized by professional chemists and laboratories for documenting their work are acceptable for documentation in laboratories contracted by DAC for damage assessment work.

All sample data, laboratory observations, and calculations will be recorded in logbooks or in bench sheets. Each lab document should, on its first page, reflect the project number, date of composing, names of analysts and assistants participating, and any other information concerning the identity of the sample analyzed. Any document reflecting results of analysis should have similar identifying data on the first page. Charts or printouts from instrumentation, graphs, and similar "display" type documents should have similar identifying information affixed to them. Both draft and smooth copies of any such documents should be retained as part of the lab documentation.

Correspondence, report notes, methods, references, sample inventories, checkout logs, etc. should be part of the permanent lab records.

Any logbook or bench sheet needs to contain (1) clear identification of who made what entries on the same, and (2) information sufficient to enable the entry-maker to recall and describe each step of the analysis performed. The analyst and his/her assistants may be called upon to testify in subsequent legal proceedings about the analysis performed and results obtained. In addition, procedures followed if it became necessary for that to be done. Irregularities (if any) observed during the analytical process should be noted. If, in the professional judgement of the analyst, a deviation from a particular analytical method is advisable or used, the deviation should be described and the reasons for that deviation should be recorded.

Logbooks or comparable permanent records must be kept which reflect each instance in which lab instruments or instrumentation is calibrated. Such records are the very foundation of the later analytical work performed, and are critical in any later legal proceeding.

Any continuous monitoring records (e.g. charts showing temperatures of storage cabinets where some samples are stored) should be kept for some years.

Before a final lab report is sent out, the lab personnel will assemble and cross-check information on sample tags, custody records, bench sheets, analysts' logbooks and/or notes, and in any relevant permanent records to ensure that

data pertaining to each particular sample is consistent throughout all lab documents.

APPENDIX 11

Evaluating Expert Assessor Training Using Fisher's Exact Test

Following training, it may be necessary to validate the performance of the expert assessors before beginning evaluations of the samples taken from the spill and control sites.

The following procedure can be applied after training or re-familiarisation of expert assessors with defects caused by petroleum contamination. It can be used with known samples, so there is an actual right or wrong answer associated with each sample. It allows the calculation of the exact probability of the results a test set of samples and shows the minimum number of samples needed for the selected level of p = 0.999 for testing of significance. The method used is Fisher's Exact Test as described by Hays (1994)[1].

The null hypothesis for the test is that the data is random in nature and the alternate hypothesis is that it is not. The probability calculated is then the indication of the chance that the results given by that assessor were only random in nature.

The data is recorded in a table as shown below.

Known Condition of Samples	Assessor's Responses		
	Taint present	Clear (no taint present)	Total
Tainted samples	a	b	a + b
Clear samples	c	d	c + d
Total	a + c	b + d	N

This is essentially finding the probability that the particular result or any result less likely is due to chance. This is calculated using the following formula and may be done using a hand-held calculator or by programming the calculation into an Excel spreadsheet.

$$p \text{ (obtained table)} = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!*}{(N!\,a!\,b!\,c!\,d!)}$$

For example, if the expert assessors are presented with 40 samples (20 clear controls and 20 exposed to taint) and the following data are obtained

Known Condition of Samples	Assessor's Responses		
	Taint present	Clear (no taint present)	Total
Tainted samples	17	3	20
Clear samples	1	19	20
Total	18	22	40

$$p \text{ (obtained table)} = \frac{(20)!\,(20)!\,(18)!\,(22)!}{(40!\,17!\,3!\,1!\,19!)} = 2.011 \times 10^{-7} \text{ (or } 0.0000002011)$$

[1] Hayes, William L. 1994. Statistics. Harcourt Brace College Publishers, New York. pp. 863-865.

In order to evaluate the results, a "cut-off" of an acceptable probability of chance must be selected. In this case, p = .001 (or 1.0×10^{-3}) is being used. This means there is less than 1 chance in 1000 that the results are due to chance and the ability of the expert assessor to evaluate the samples is validated. The results in the example show 2 chances in 10 million that the results are due to chance (or 1 chance in 5 million).

The following gives examples of data collected and the calculations based on each. These examples show that if a minimum number of 20 samples are used (10 clear and 10 tainted), the pattern of results (calculated as shown in the above tables) gives assurance at P<0.001 that the expert assessor can correctly identify taint when it is present in the samples. If more samples are used, the formula given above can be used to calculate the results for any data set.

Example 1.

Known Condition of Samples	Assessor's Responses		
	Taint present	Clear (no taint present)	Total
Tainted samples	9	1	10
Clear samples	1	9	10
Total	10	10	20

p (obtained table) $= \frac{(10)!\,(10)!\,(10)!\,(10)!}{(10!\ 9!\ 1!\ 1!\ 9!)} = 0.00054$

Example 2.

Known Condition of Samples	Assessor's Responses		
	Taint present	Clear (no taint present)	Total
Tainted samples	8	2	10
Clear samples	0	10	10
Total	8	12	20

p (obtained table) $= \frac{(10)!\,(10)!\,(8)!\,(12)!}{(10!\ 8!\ 2!\ 0!\ 10!)} = 0.00036$

Example 3.

Known Condition of Samples	Assessor's Responses		
	Taint present	Clear (no taint present)	Total
Tainted samples	10	0	10
Clear samples	2	8	10
Total	12	8	20

p (obtained table) $= \frac{(12)!\,(8)!\,(10)!\,(10)!}{(10!\ 0!\ 10!\ 2!\ 8!)} = 0.00036$

Explanatory Note on the use of Factorial Notation: The notation "!" means the factorial of the number, e.g. 4! = 4 x 3 x 2 x 1 = 24. When using a hand-held calculator, the usual algebraic "cancelling" of numbers in the numerator can be done and then the

value for *p* calculated. It must be remembered that, e.g. 20! divided by 17! would leave 20 x 19 x18 in the numerator and 20! / 19! would leave 20 in the numerator, etc. This does not have to be done if the calculation is programmed into Excel.