Part II: Section IV

Authorization and Control of Analysis and Sampling Entities and Samplers

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CHAPTER I. AUTHORIZATION AND CONTROL

1. REQUIREMENTS FOR THE AUTHORIZATION AND OPERATION OF ANALYSIS ENTITIES, SAMPLING ENTITIES, AND SAMPLERS

1.1 GENERAL PROCEDURE FOR THE AUTHORIZATION OF ANALYSIS ENTITIES

To become part of the Export Fishery Products Certification System of SERNAPESCA, in the area of analysis, the laboratories must comply with the following general requirements:

1.1.1 REQUIREMENTS

1) The laboratories must guarantee their independence from organizations to which they provide services.
2) The laboratories must ensure confidentiality and impartiality in the results of the analyses.
3) The laboratories must work with qualified staff to carry out the analyses for which they are applying.
4) The laboratories must have the proper equipment, supplies, and reagents for the task to be conducted, as per the requirements set forth in the corresponding document for the relevant area of the laboratory applying for authorization.
5) The laboratories must use SERNAPESCA's official analysis methods, as available. In this respect, the laboratories must have at least one original version of the Chilean Standard or ISO, as appropriate.
6) The laboratories must have the accreditation for Testing Laboratories provided by the National Institute of Standardization (INN), as per NCh ISO 17025, and specifically under the INN-SERNAPESCA agreement.
7) The laboratories must be recognized as a Public Health Food Science Laboratory, as per Decree 707 of 1999, of the Ministry of Health.
8) The physical-chemical, organoleptic and microbiological analysis methods of the Pharmaceutical Products, Contaminants, Prohibited Substances and Unauthorized Substances Residues Control Program and of the Bivalve Mollusks Sanitation Program (microbiological, pesticides, halogenated organic compounds, heavy metals, phytoplankton, marine toxins), must be verified or validated, as the case may be, and based on what is set forth in Chapter III, and on the European Commission Decision 657/2002, as appropriate.
9) The analysis methods considered within the Sanitation Operational Procedures (SOP) of the Quality Assurance Program (QAP) must only have the corresponding accreditation under the INN-SERNAPESCA or the INN-SISS agreement, as appropriate. This includes all the methods required by the European standards for water, as described in Chapter III, Items 2.19 and 6.20. The above means that the laboratory does not require the authorization of SERNAPESCA to conduct these analyses, subject to the compliance with the previously described requirements.
10) The entities that wish to issue reports in digital format must comply with what is described in Exempt Resolution No. 102 of January 21, 2013, published in the Official Gazette of the Republic of Chile on January 31, 2013, which regulates the use of the Electronic Signature for these type of reports, which states the requirement to have an Advanced Electronic Signature. For this, the entity must request authorization to the National Directorate of SERNAPESCA, informing the way to prove its validity and authenticity, as well as the authorized personnel for these purposes.

11) The laboratories that conduct the analyses for parasite confirmation will only require the authorization of SERNAPESCA.

1.1.2 REQUIRED DOCUMENTATION

When applying, the laboratory must present the following:

a) General Documentation:

- Letter of request to become part of the SERNAPESCA Laboratories Program, addressed to the Foreign Trade Deputy Director of SERNAPESCA, indicating the corresponding area of analysis. This letter, as well as all the documentation related to the application request, must be signed by the legal representative.
- Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).

b) Legal Documentation:

- Sworn Declaration subscribed before a Notary Public (Part III, Annexes, Chapter II).
- Photocopy of the articles of incorporation and their corresponding amendments, if any, and a certificate of good standing issued by the Competent Authority. When applying, this certificate must not be older than 60 calendar days. The purpose of the company must include the provision of laboratory and/or technical analysis services, which must be included in the articles of incorporation or deed for amendments.
- A copy of the valid certificate of incorporation with all marginal notes provided by the Real Estate and Commerce Registrar dated no older than 90 calendar days.
- A document that accredits the legal capacity of the legal representative to act on behalf of the legal person, and certificate of subsistence of the legal representative's powers. When applying, this certificate must not be older than 60 calendar days.
- A copy of the Legal Representative's RUT (ID number).
- A copy of the Company's (Taxpayer's ID).

c) Technical Documentation:

- A copy of the Testing Laboratory Accreditation Certificate issued by the National Institute of Standardization under NCh ISO 17025.
- The scope of the accreditation under the INN-SERNAPESCA agreement, which describes the determinations that were accredited for export fishery products, with methodologies in compliance with the official requirements of SERNAPESCA.
- A copy of the Recognition as a Public Health Food Science Laboratory, as per S.D. 707/1999 of the Ministry of Health.
- Controlled copy of the Quality Manual, written based on the recommendations of NCh-ISO 17025.
- Validations and information concerning the techniques to be included in the application for becoming an Analysis Entity authorized by SERNAPESCA:

Area of Microbiological Analysis:

- A copy of the method implemented by the laboratory. It must include the description of the media and a list of supplies and equipment.
- Statistical data of repeatability and reproducibility that provides information on the verification of the method in the laboratory, which must include all the analysts applying the method.

Area of Chemical Analysis:
This area includes instrumental methods whose purpose of analysis is a chemical substance. The documents to be presented are:

- A copy of the method implemented by the laboratory. It must include a description of the reagents and a list of supplies and equipment.
- A copy of the validation or verification method for the methodology, as appropriate, which must list the international standards on which it is based, among which the European Commission Decision 657/2002 is required.
- Summary of the validation or verification of the method, as appropriate, which must include the statistical parameters obtained by the laboratory, that is:
  - Specificity.
  - Limit of Detection and Quantification.
  - Calibration Curve (mathematical description and work interval).
  - Veracity (with Certified Reference Material or Recovery).
  - Precision (Repeatability and Reproducibility).
  - Robustness of the Method.
  - Uncertainty.
- Information that supports the acquisition of the statistical parameters obtained in the previous item (chromatograms, absorbencies, volumes, calculations, etc. as appropriate).
- List of equipment and supplies required for the analyses included in the scope of the accreditation, specific for SERNAPESCA, as required in the Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).

Once the information has been reviewed, an inspection visit in charge of the SERNAPESCA inspector of the jurisdiction of the laboratory will take place, and its purpose is to verify that the analysis methods, analytical information, and other procedures are conducted in compliance with the requirements set forth by the National Fisheries and Aquaculture Service.

1.2 SPECIFIC PROCEDURES FOR THE AUTHORIZATION OF ANALYSIS ENTITIES

To become part of the Export Fishery Products Certification System of SERNAPESCA, except as otherwise provided herein, the laboratories must comply with the General Procedure for the Authorization of Analysis Entities, set forth in Item 1.1. herein, and must also comply with the specific requirements described according to the type of authorization to which they are applying.

1.2.1 FOOD SCIENCE LABORATORIES

a) Service Laboratories: These type of laboratories do not have any additional specific requirements to those described in Item 1.1.

b) SERNAPESCA Verification Laboratory: In addition to the requirements set forth in Item 1.1, the laboratories must comply with the following:

- They must only conduct analyses of export fishery products exclusively for SERNAPESCA.
- They must be directed by a professional with a university degree in biology or chemistry, with at least 5 years of experience in the specific analysis area.

Once the compliance with the previous requirements is accredited, and once the agreement with the Service is subscribed, the laboratory will be understood as authorized to provide the analysis services described in the scope.

c) University Laboratories for the Analysis of Marine Biotoxins

In addition to the requirements set forth in Item 1.1., the laboratories must comply with the following:

- A valid recognition for carrying out biotoxins analyses, provided by the Public Health Institute (ISP).
- The proper equipment, supplies, and reagents for the tasks to be carried out, as per the requirements set forth in Chapter III, Item 3.
- The analysis methods employed must correspond to official methodologies of SERNAPESCA, as set forth in Chapter III, Item 3.

d) Fish Processing Plant Laboratories

In addition to the requirements set forth in Item 1.1., the laboratories must comply with the following:

- The laboratory may only carry out analyses for the fish processing plant to which it belongs. It may not provide analysis services to third parties.
- The plant's QAP must be validated by SERNAPESCA.

1.2.2 PHYTOPLANKTON ANALYSIS LABORATORIES

In addition to the requirements set forth in Item 1.1., the laboratories must comply with the following:

a) They must be directed by a professional with a university degree in the field of biology or chemistry, with experience in phytoplankton analyses.
b) The proper equipment, supplies, and reagents for the tasks to be carried out, as per the requirements set forth in Chapter III, Item 5.

1.3 AUTHORIZATION GRANTED TO LABORATORIES

A laboratory that is accredited by the INN, and is recognized by the Public Health Institute, and complies with all the aforementioned requirements, may request to become part of the List of Entities Authorized by SERNAPESCA, which must be formalized through a letter addressed to the Foreign Trade Deputy Director together with all the required documentation.

1.4 PROCEDURE TO AUTHORIZE A NEW ANALYSIS

1.4.1. FINAL AUTHORIZATION

The Laboratories that request the authorization of a new analysis from SERNAPESCA, and that are accredited by the INN and/or are recognized by the Ministry of Health, must consider the following:

- Request the authorization of such analysis under the framework of the SERNAPESCA Laboratories Program through a letter or email addressed to the Foreign Trade Deputy Director with a copy to the Regional Directorate of the corresponding jurisdiction.
- Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).
- All the information concerning the analysis technique must be attached to the application, as outlined in Item 1.1.2.c. herein.

The National Directorate of SERNAPESCA will assess the information and will request an inspection visit in charge of the regional inspector, whose purpose is to confirm that the analysis method, the

analytical information, etc. are conducted in compliance with the requirements set forth by the National Fisheries and Aquaculture Service.

Finally, the National Directorate of SERNAPESCA will send the corresponding Authorization fax to the laboratory.

1.4.2. TEMPORARY AUTHORIZATION

Those laboratories that belong to the SERNAPESCA network may obtain a temporary authorization, granted that they request the authorization for a new analysis that is not accredited by the INN, as long as this extension does not imply the creation of a new area of analysis, which is specified in Item 2 of the Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).

The requirements and documentation described in Items 1.1.1 and 1.1.2 herein, must be considered together with the documentation that attests the accreditation process with INN, as the case may be.

SERNAPESCA reserves the right to revoke this authorization in case the information that attests the accreditation process is not presented promptly or if no progress is made in the process.

1.5 RESPONSIBILITIES DURING THE AUTHORIZATION

Through this authorization, the entity understands its responsibility as part of the export fishery products certification system, and it commits to:

a) Comply with this Manual and the specific instructions provided by this Service.
b) Obtain information every month on the amendments made to this Manual, with the purpose of keeping its work procedures up to date.
c) Provide a monthly report to SERNAPESCA's Foreign Trade Sub-Directorate with statistics on the analyses conducted in the laboratory for final product control (FPC), pre-harvest residues analyses (PHA) and of the Bivalve Mollusks Sanitation Program (BMSP). The *Excel* version for the "Analysis Statistics Spreadsheet" can be downloaded in Part III, Chapter II of the Food Safety and Certification Manual, available at Sernapesca's website.
d) Send the results of the Biweekly QAP Verifications to SERNAPESCA's Foreign Trade Sub-Directorate to the following email address: verpac@sernapesca.cl, and the results of the Official QAP Verifications to the following email address: veroficial@sernapesca.cl.
   Those above must be reported through the *Excel* spreadsheets "Verification Results Report" and "SERNAPESCA Verifications Results Report" available in Part III, Chapter II of the Food Safety and Certification Manual at the Service's Website.
   The reported analyses must be named according to the "Analysis Name" document available in Part III, Chapter II of the Food Safety and Certification Manual.
e) Participate in the activities required by the Service, such as:
   - Fitness for purpose tests and quality assessment programs, among which those organized by SERNAPESCA in conjunction with another competent national or international entity are considered mandatory. For these purposes, SERNAPESCA will be entitled to request the provider of the fitness for purpose tests, the participation codes of the laboratories authorized by the Service, as well as any additional information that it deems necessary for assessing the competency of the laboratory
   - Audits arranged by competent foreign authorities.
f) Participate in proficiency tests, in such a way that at least 1 proficiency test per year is included, per authorized area of analysis and at least 1 per authorized analysis every 4 years.

Whose program and results should be reported periodically to the National Directorate of SERNAPESCA.

g) Use Certified Reference Material, at least monthly, within the analyses quality control, as long as they are available in the market. In the particular case of metals analyses, this is mandatory.

h) Marine biotoxins analysis results must be informed with their respective uncertainty, as it affects compliance with the limits established for toxins, outlined in Section I and III of this part of the manual.

i) Provide all the required information promptly.

j) Inform, with anticipation, to SERNAPESCA, of any changes related to the activities carried out in the scope of the Export Fishery Products Certification System, such as changes in infrastructure (renovations, moving to other facilities, among others), equipment, supplies, staff, results report format (in paper or in digital format). Any necessary documentation will be required depending on the implications of these changes.

k) In the specific case of changes in infrastructure and equipment directly related to the analyses conducted for SERNAPESCA, the documents to be presented must be the following:
   - The letter that informs and describes the changes that will take place.
   - Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II). Only in the case of changing the address.
   - Gantt chart with the schedule for the change, including the period in which the laboratory must outsource (out-task or send out) the analyses affected by the change.
   - INN document that proves the approval of the change, if applicable.
   - Control spreadsheets for the equipment and instruments involved in the change; before and after it.
   - Records that prove that the performance of the methodologies involved in the change, including those analysts conducting them, has been kept.
   - Send a controlled and updated copy of the Quality Manual, every time that it has been amended, to the corresponding Regional Office.
   - Allow the access of SERNAPESCA staff to the Entity's facilities, so as to carry out inspections.
   - Changes related to the legal structure of the entity:

l) If there is a change in the legal person, the following documentation must be presented in addition to that required in the Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).
   - An authorized copy of the public deed (SRL) or reduced to a document of public record (S.A.) that accredits the modification.
   - A copy of the certificate of incorporation in the Commerce Registrar.
   - Proof of Incorporation valid for 90 days.
   - Photocopy of the notarized public deed that accredits the power of the legal representative.
   - Photocopy of the Company's RUT.
   - Photocopy of the legal representative's RUT.
   - Letter of relinquishment of authorization from the legal person (Part III Annexes, Chapter II).
   - Updated sworn declaration (Part III Annexes, Chapter II).

m) Change of Manager or Administrator:
   - An authorized photocopy of the public deed (SRL) or reduced to a document of public record (S.A.) that accredits the appointment of the manager or administrator.
   - A copy of the Minutes of the Meetings of the Board of Directors (S.A.), which proves the powers of the legal representative to act on behalf of it, or information pertaining the powers of the legal representative if they are not included in the articles of incorporation (SRL).
   - Proof of Incorporation valid for 90 days.

- Photocopy of the Company's RUT.
- Photocopy of the legal representative's ID card.
- Updated sworn declaration (Part III Annexes, Chapter II).

n) Analyses (subcontracting or sending out) must only be outsourced between laboratories authorized by SERNAPESCA, so as to comply with what is outlined in this Manual regarding the integrity of the sample, the terms established between the sampling and the analysis and the terms to inform results. The agreement subscribed by the parties must clearly refer to the responsibility before SERNAPESCA to inform about statistics, unfavorable results and the issuance of analysis reports. Regarding the latter, it must be noted that both the laboratory outsourcing the services, as well as the one conducting these outsourced services, must issue a Results Report. The outsourcing laboratory must issue a Results Report that compiles all the analyses, this is, those that it conducted as well as those outsourced, the laboratory conducting the outsourced analyses must issue a results report with all the analyses conducted for the outsourcing laboratory.

o) If the laboratory is not required to conduct an analysis authorized by the Service, it must prove that it keeps its competency by carrying out this analysis through a fortified sample, a comparison with another laboratory or a fitness for purpose test at least once a year, which must be informed to SERNAPESCA sending the supporting information that demonstrates their proper execution.

1.6 TERMINATION OF AUTHORIZATION

The Service may terminate the authorization granted to an entity due to non-compliance with the requirements outlined in this Manual, as well as for improper actions, that determine the loss of trust and confidence. Item 2 herein provides more information on termination of authorization.

1.7 PROCEDURE TO AUTHORIZE SAMPLING ENTITIES

To become part of SERNAPESCA's Export Fishery Products Certification System, in the area of Export Fishery Products and of the Bivalve Mollusks Sanitation Program, the entities must comply with the requirements outlined in this Chapter, in those specific cases where what is outlined applies to this activity, in addition to the following:

a) The sampling entities must guarantee their independence from organizations to which they provide services.

b) They must ensure confidentiality and impartiality in the procedures that they conduct.

c) They must be accredited by the National Institute of Standardization (INN) under standard NCh-ISO17020:2012 "Compliance assessment - Requirements for the operation of different types of bodies that conduct the inspection".

d) Those entities that obtain samples to be analyzed in the same laboratory must include in their scope their accreditation for NCh-ISO17020:2012, item 5.7 regarding "Sampling".

e) This requirement applies to all new Entities that request authorization from August 2008 and all Entities currently authorized from February 2010.
   - The proper equipment, supplies, and reagents for the tasks to be carried out, as per the requirements set forth in Chapter III, Item 2, corresponding to the area of authorization of the Entity.

f) Employ the official SERNAPESCA sampling methods as available.

g) Establish work procedures as set forth in Chapter II, Item 1.

h) Have a technical manager in charge of the area, with technical qualification of at least 4 semesters of higher education in the area of biology, chemistry, food, aquaculture or related. In case of being a technician from another area, he/she must accredit a minimum of two years'

experience in Export Fishery Products (EFP) and/or the Bivalve Mollusks Sanitation Program (BMSP) sampling activities, as the case may be.

i) To start the application process, the interested Entities must:

- Send a letter of request to become part of the SERNAPESCA Laboratories Program, addressed to the Foreign Trade Deputy Director, stating their intention to become part of the SERNAPESCA Fishery Products Certification System, as a Sampling Entity.
- Attach the Application form for export fishery products analysis, sampling and physical organoleptic evaluation entities (Part III, Annexes, Chapter II).
- The entities must work with qualified staff to carry out the sampling tasks for which they are applying.

Once the information has been evaluated, the National Fisheries Director will reply via certified letter.

1.8 PROCEDURE TO AUTHORIZE SAMPLERS

To become part of the SERNAPESCA Export Fishery Products Certification System (EFP) or the Bivalve Mollusks Sanitation Program (BMSP), the interested parties must comply with the following requirements.

1.8.1 GENERAL PROCEDURE FOR THE AUTHORIZATION OF SAMPLERS

Those Samplers included in the Official List of SERNAPESCA, are those to which the Service delegates functions and responsibilities to conduct Samplings of Export Fishery Products (EFP) and/or of the Bivalve Mollusks Sanitation Program (BMSP).

a) Requirements:

To obtain the authorization to conduct the Sampling of Export Fishery Products or for the Bivalve Mollusks Sanitation Program, in addition to passing the corresponding Official Course, the interested parties must:

- Have a diploma or certificate of graduation from technical-professional secondary education, technician of higher education of at least 4 semesters, or professional education, in the area of biology, chemistry, food, aquaculture or related. If necessary, have a document that accounts for the rate of progress of the curriculum.
- Be supported by an entity that is authorized by SERNAPESCA in the area of sampling.
- Have the authorization from the parties, in case the interested party requests to provide its services for more than one entity; this must be stipulated in a letter issued by each one of them stating their agreement.
- Present a sworn declaration subscribed before a notary public, which states that there are not any conflicts of interests in the sampling activity to be conducted.

b) Application:

The interested parties must comply with the requirements set forth in Item 2.1 of this Chapter and send the following documents to the Central Office of SERNAPESCA:

- A Sponsorship Letter from the Sampling Entity supporting it.
- Updated curricular information.
- Photocopy of the technical or professional diploma with at least 4 semesters of higher education studies.
- Two passport size photos (3 x 4 cm) in photo paper, light background, with full name and RUT.

c) The validity of the authorization:

The authorization for Samplers will be valid for 10 years, from the date of approval of the course. The authorization will expire after this period, and the Sampler must renew it.

This renewal must take place during the calendar year of its expiration, as outlined in Item 1.8.1.d.

Notwithstanding the aforementioned, the Service may terminate the authorization due to non-compliance with the requirements and procedures established to conduct the sampling activity, or due to improper actions, that determine the loss of confidence reposed.

d) Authorization Renewal:
Those Samplers interested in renewing their authorization must comply with the following requirements:

- The Sampling Entity that supports it must send a request for renewing the Sampler authorization to the National Directorate of SERNAPESCA.
- Take and pass again the Official Course for Sampling of Export Fishery Products or for the Bivalve Mollusks Sanitation Program, as appropriate.
- The test will be graded as per Item 1.10.3.d of this Chapter.

1.9 PROCEDURE TO AUTHORIZE SAMPLING OFFICIAL TRAINERS

Official Trainers are those persons authorized by SERNAPESCA to teach the Official Export Fishery Products (EFP) and the Bivalve Mollusks Sanitation Program (BMSP) Courses.

1.9.1 REQUIREMENTS

To apply to obtain the authorization of SERNAPESCA as Trainers for Export Fishery Products or for the Bivalve Mollusks Sanitation Program, the interested professionals must comply with the following requirements:

a) If applying to become an Export Fishery Products Sampling Trainer, the professional must have university training, be a graduand or graduate, with at least 8 academic semesters of higher education in the area of basic sciences, food science, fishery, aquaculture or similar, in addition to having knowledge of microbiology and statistical quality control.
b) In the case of trainers for the Bivalve Mollusks Sanitation Program, their education must be in the area of aquaculture, marine science or similar, in addition to having knowledge in microbiology, and physical and biological oceanography.
c) Accredit with experience and knowledge its relationship with food products samples collection, when the person is applying to become an Export Fishery Products Sampling Trainer. To apply for the authorization to work as a Trainer for the Bivalve Mollusks Sanitation Program, experience and knowledge in the collection of BMSP samples must be accredited through the specialization course as outlined in letter a) of this Item.
d) At least 5 years of experience in quality control and fishery products sampling must be accredited. For the case of the Bivalve Mollusks Sanitation Program, the accredited experience must be in the in the area of BMSP monitoring programs.
e) Accredit academic or teaching experience in the subject.
f) Pass the Course for Sampling of Export Fishery Products or for the Bivalve Mollusks Sanitation Program, as appropriate.

1.9.2 APPLICATION

Those who are interested in applying must send a letter of request to the Head of the Foreign Trade Sub-Directorate of the National Directorate, attaching the corresponding curricular information and indicating the position of Official Trainer to which they are applying.

If the application is accepted, the professional will be notified and incorporated to the Registry of Authorized Trainers for Sampling of Export Fishery Products or the Bivalve Mollusks Sanitation Program, as appropriate.

1.9.3 VALIDITY OF THE AUTHORIZATION

To keep authorizations current, all Trainers must teach at least one Official Course per year. This Course will be open to all Sampling Entities authorized by SERNAPESCA.

1.9.4 TERMINATION OF THE AUTHORIZATION

The Service may terminate the authorization granted to a professional due to non-compliance with the requirements outlined in this document, as well as for improper actions, that determine the loss of trust and confidence.

1.10 OFFICIAL COURSE FOR SAMPLING EXPORT FISHERY PRODUCTS OR FOR THE BIVALVE MOLLUSKS SANITATION PROGRAM

Trainers will be in charge of planning, teaching and providing all the necessary material to carry out the Course, as outlined in this document. SERNAPESCA will be in charge of designing the test and its evaluation.

1.10.1 AUTHORIZATION TO CONDUCT THE COURSE

The Trainer must send a letter informing the opening of the course at least 60 days in advance to the National Directorate with a copy to the Regional or Provincial Office of the National Fisheries Service of its jurisdiction, also attaching the following information:

a) Date and location of the Course.
b) Name of the trainer in charge of the Course.
c) Estimated number of participants.
d) Course program, according to the contents described in 1.10.3.a or 10.1.4.a of this Chapter.
e) If there are any guest speakers, their CVs must be attached, also indicating the subject matters to be addressed. Guest speakers must not use more than 50% of the course.
f) Communication model through which the course will be promoted.

SERNAPESCA will have 5 days to respond once the application has been received.

The Trainer must confirm the opening of the course to the Regional Office of SERNAPESCA, with a copy to the National Directorate, 15 days before its start date.

An authorized course may be postponed at the request of the Official Trainer only once, with at least 15 days before its start date.

1.10.2 REQUIREMENTS TO CONDUCT THE COURSE

Official Trainers must comply with the following requirements:

a) The Trainer must be present during the entire Course.

b) The course's program will be based on the contents described in 1.10.3.a or 10.1.4.a of this Chapter.
c) The Trainer will be responsible for verifying that those attending the course comply with all necessary requirements, as outlined in Item 1.8.1.a.
d) Once the course has been conducted, the trainer must send a list of participants with their RUT and the entity to which they belong to the Service.

SERNAPESCA will reserve the right to attend the course. However, it will always supervise the final evaluation.

1.10.3 COURSE FOR EXPORT FISHERY PRODUCTS SAMPLERS

a) Course Program

The course program will be developed by the Trainer, considering the following content:

1) Basic concepts of microbiology (minimum time 30 minutes):
- Definition and characteristics of microorganisms.
- Factors that interfere in the multiplication of microorganisms.
- Pathogenic and altering microorganisms:
  - *Salmonella*
  - *Listeria monocytogenes*
  - *Escherichia coli*
  - *Vibrio parahaemolyticus*
  - *Staphylococcus aureus*

2) NCh 43 Standard on random collection of samples (minimum time 1.5 hours)
- Application of the standard:
  - Sample collection from a lot.
  - Sample collection from a series of lots.
  - Sample collection from a consecutive series of lots.
  - Systematic sampling.
  - Exercises.

3) National Fisheries Service (minimum time 4 hours):
- The role of the Service in the certification of export fishery products. Legal framework. Importance and responsibility of the Sampler authorized by SERNAPESCA.
- Definitions.
- Sanitary certification, which must consider the following:
  - Background information.
  - Definitions.
  - Procedures and technical requirements to authorize notifications of shipments for export fishery products.
  - Procedures and technical requirements for the issuance of the sanitary certification.
  - Procedures and technical requirements for the issuance of the certification of origin.
  - The backup file of notifications and certificates.
  - Rates.
  - Provision of blank certificate forms.
  - Fishery standards control.
  - Modifications taking place after export.

4) Certification as per the Certification Program (Section III):

- Certification as per the Quality Assurance Program (Section II).
- Residues Control Program (Section I, Chapter II).
- Basic concepts of the sensory examination, as per Section IV, Chapter II, Item 1, Section IV, Chapter II, Item 3 and Section III, Chapter IV, Item 1.

5) Sampling and Inspection of Export Fishery Products (minimum time 5 hours):
- Authorization of Sampling Entities (Section IV, Chapter I, Item 1).
- Administrative Procedures for Sampling Entities (Section IV, Chapter II, Item 1).
- Sampling for the Certification Program.
- Sampling for the Quality Assurance Program.
- Sampling for SERNAPESCA to verify the Residues Control Program (Section I, Chapter II).
- Sampling of fish in farms for the Residues Control Program (Section I, Chapter II).
- Inspection for the Imports Control Program (Part II, Section V).
  Inspection and sampling procedure for export fishery and aquaculture products shipments with destination to the Eurasian Economic Union (EEU). (Section IV, Chapter II, Item 1 and Section II, Chapter IV).

6) Sampling procedures, Section IV, Chapter II, Item 2 (minimum time 4 hours):
- Scope and field of application.
- Definitions.
- Supplies.
- Collection of the sample.
- Fish meal sampling.
- Oil sampling.
- Fish sampling in farms.
- Seaweed sampling.
- Samples labeling.
- Transportation and storage of samples.
- Collection of counter samples.

7) Water sampling (minimum time 1 hour)
- Directive 98 / 83 / EC on the quality of waters intended for human consumption.
- NCh 409/2 Sampling.
- SISS Manual, Chapter 1 - Packaging and preservatives.
- Sampling Methodology.
- On-site pH and temperature measurement.

8) Support materials.
- The documents of Section III, Chapter IV, items 1 and 2, Section IV, Chapter II, Item 2 and Section I, Chapter II.

During the evaluation, the student may only use a summary chart of the physical organoleptic characteristics that live and chilled-refrigerated products must comply, as set forth in Section II, Chapter V, Item 1.

9) Case discussion (minimum time 4 hours).
At least one case per each type of sampling must be considered, that is to say, the end product, monthly and annual water and of handlers and surfaces.

b) Duration
As indicated by the Trainer conducting the course, considering a minimum of 24 hours.

c) Official Course Test
The student must take a written test, which will be given by a SERNAPESCA Inspector. SERNAPESCA will inform the corresponding entities, within 15 days, of the results of the test.

The minimum passing score is 75% of the total score of the test.

Those students that score more than 70% and that do not achieve a minimum passing score, may request a repeat test, which must be passed with a minimum score of 75%.

d) **Authorization Renewal Test**
The passing score must be 75% of the total of the test score. Those students that obtain more than 70% but do not reach the minimum passing score, may request a repeat test, which must be passed with a minimum score of 80%.

e) Participants Authorization Procedure
The authorization of the sampler will be informed via fax to the Sampling Entity that has requested its authorization, and it will be accredited through the SERNAPESCA credential, without prejudice that the organizers of the course provide a certificate of participation. Such credential will be sent by the Central Office of SERNAPESCA, via certified letter, to the address of the corresponding Sampling Entity.

Those that have obtained the required scores will be incorporated to the Official List of Samplers Authorized by SERNAPESCA.

1.10.4 COURSE FOR SAMPLERS OF THE BIVALVE MOLLUSKS SANITATION PROGRAM

a) Course Program

The course program will be developed by the Trainer, considering the following sections of the manual: Section I, Chapter I, Section IV, Chapter II, Item 2, Section IV, Chapter II, Item 1 and Regulation (EC) No 854/2004:

1) Basic concepts of microbiology (minimum time 2 hours):
- Definition and characteristics of microorganisms.
- Factors that interfere in the multiplication of microorganisms.
- Pathogenic and altering microorganisms in bivalve mollusks.

2) General concepts of marine toxins and their effect on public health (minimum time 2 hours).

3) General concepts of harmful algal blooms (minimum time 2 hours).

4) Aspects relative to safety on ships (minimum time 1 hour).

5) National Fisheries and Aquaculture Service (time 2 hours):
- The role of the Service in the certification of export fishery products. Legal framework. Importance and responsibility of the Sampler authorized by SERNAPESCA.
- Definitions.

6) Application of BMSP monitoring programs (minimum time 2 hours):

- Authorization of Samplers (Section IV, Chapter I, Item 1).
- Administrative Procedures for Samplers (Section IV, Chapter II, item 1), in the framework of the BMSP.
- Request and handout of BMSP forms.

7) Sampling procedures (Section IV, Chapter II, Item 2, minimum time 3 hours):
- Scope and field of application.
- Definitions.
- Supplies.
- Flesh/meat sample collection.
- Sample sizes.
- Phytoplankton sampling methodology.
- Samples labeling.
- Transportation and storage of samples.

8) Basic concepts of the BMSP (minimum time 3 hours):
- Classification and Monitoring.
- Parameters to be evaluated.
- Classification criteria.
- Application of contingency plans.

9) Coastline evaluation (minimum time 4 hours):
- Definitions.
- Identification and classification of sources of contamination.
- How to conduct a coastline inspection.
- Minimum contents of a coastline report.

10) On-site activity (minimum time 8 hours, with SERNAPESCA):
- Walk around the coastline in an extraction area.
- Marine tour with a hands-on activity of qualitative and quantitative phytoplankton sampling.

b) Duration

As indicated by the Trainer conducting the course. Minimum of 48 hours.

c) Official Course Test

The course evaluation consists of 3 parts:
- Written test based on multiple choice and essay questions (50%).
- Skills evaluation on on-site activity (30%).
- On-site activity report (20%).

The on-site activity report must be written on the same day of the written theoretical evaluation.

The score to pass the course is 75%. Those students that score more than 70% and that do not achieve a minimum passing score, may request a repeat test, which must be passed with a minimum score of 75%.

SERNAPESCA will inform the corresponding entities, within 15 days, of the results of the test.

d) Authorization Renewal Test

The passing score must be 75% of the total of the test score. Those students that obtain more than 70% but do not reach the minimum passing score, may request a repeat test, which must be passed with a minimum score of 80%.

e) Participants Authorization Procedure

The authorization of the Sampler will be informed via fax to the Sampling Entity that has requested its authorization, and it will be accredited through the SERNAPESCA credential, without prejudice that the organizers of the course provide a participation diploma. Such credential will be sent by the Central Office of SERNAPESCA, via certified letter, to the address of the corresponding Sampling Entity.

Those that have obtained the required scores will be incorporated to the Official List of Samplers Authorized by SERNAPESCA.

2. PROCEDURES RELATED TO THE INSPECTION AND CONTROL OF ANALYSIS AND SAMPLING ENTITIES AUTHORIZED BY THE NATIONAL FISHERIES AND AQUACULTURE SERVICE

The purpose of this section is to establish the procedures, to control the activities delegated to Sampling and Analysis Entities and Samplers, according to requirements for the type of authorization.

2.1 PROCEDURES RELATED TO THE INSPECTION OF ENTITIES

2.1.1 GENERAL PROCEDURES RELATED TO THE INSPECTION OF ENTITIES

All Entities to which SERNAPESCA delegates functions, must be regularly inspected.

a) Those inspections must not be announced, except for exceptional cases.
b) The inspections will consist of three stages:
   - Kickoff meeting, as appropriate, where the purpose of the visit will be described to the interested party.
   - Inspection process. Carried out according to a guideline or instructions where the facilities and/or activities are reviewed and checked, and the relevant staff is interviewed until being able to determine the level of compliance with the Standards.
   - Final meeting, as appropriate, where the results of the Inspection, the measures expected to be adopted by the entity, the deadlines to correct non-compliances or deficiencies, or other measures that the Foreign Trade Sub-Directorate may consider necessary to implement are presented.
c) The purpose of the inspections is to verify the compliance with the standards set forth by SERNAPESCA by the authorized Entity.
d) A report must be written after all inspections, and they must be properly recorded.
e) The report must be sent to the Inspected Laboratory with a copy to the Foreign Trade Sub-Directorate of the National Directorate of SERNAPESCA.
f) Reasonable deadlines must be established to implement the actions intended to correct the noncompliances and any observations from the inspection.
g) The description of non-compliances and their corresponding corrective actions, as well as the answers to the observations found, must be verified during the following inspections, according to the deadlines established.

2.1.2 SPECIFIC PROCEDURES RELATED TO THE INSPECTION OF ENTITIES

The inspection of authorized entities for the sampling and analysis of fishery products for export will be coordinated by the National Directorate, by the Authorizing and Control Coordinator of Analysis, Sampling and Sampling Entities.

The inspections may be carried out by the coordinator of the National Directorate or by the regional inspectors of SERNAPESCA.

2.1.3 INSPECTION PROCEDURES

The inspection must be conducted according to the inspection checklists established, according to the type of analysis authorized to the laboratory, or it could be conducted for a specific situation according to the needs of the moment.

After the inspection, a final meeting will be held to present the observations and non-compliances or non-conformities, detected during the visit, describing the evidence on which the findings were based.

If the inspection detects situations that call for the suspension of the authorization granted, the Inspector will contact the National Directorate via e-mail so as to evaluate the situation based on what is described in Item 2.3 of this Chapter.

2.1.4 INSPECTION REPORT

A report of the activities and non-conformities, observations or non-compliances found during the inspections must be written within 10 working days. It will be the responsibility of the Inspector in charge of the visit, who must follow the Report form for Laboratory Inspections.

Such report must be addressed to the Entity, with a copy to the National Directorate of the Service. If it is necessary to suspend the entity, the report must clearly indicate the non-conformities that lead to this decision, which will be made jointly by the National Directorate.

The report must determine the term for the implementation of corrective actions for the observations.

The inspections carried out in addition to those scheduled quarterly, due to purposes that may include the application of a new laboratory, expanding the scope of the authorized laboratory, audit from the National Institute of Standardization (INN) and the Public Health Institute (ISP), an externally detected non-compliance, among other reasons, must be informed to the laboratory via e-mail, with a copy to the National Directorate of SERNAPESCA.

The previous does not include laboratory internships, which must take place according to what is outlined in Item 2.5 of this Chapter.

2.1.5 INSPECTION FOLLOW-UP

The laboratory will have a maximum of 30 consecutive calendar days to answer the findings of the inspection report through a solution report for non-conformities and observations. The solution report for non-conformities and observations must be written based on the investigation of their causes and must describe the actions for their prevention and correction. This report will comprise the solution of non-conformities and observations or a schedule of the measures to be adopted. A summarized form of this report must be presented before the Regional Directorate of SERNAPESCA

with a copy to the National Directorate. If necessary, during this procedure, the Entity may request, by presenting justified reasons, an extension of the term set forth for the correction of non-conformities, observations, and non-compliances.

The following inspection must be initially aimed to verify on site, the implementation of the corrective actions and observations pending from the last inspection.

Any new non-conformities and observations found in the follow-up inspection and those that still have not been corrected will be informed to the Entity in order to complete the inspection, and after 10 business days, they will be informed in writing both to the laboratory and the National Directorate of the Service.

Each Regional Directorate of SERNAPESCA must keep a record of all laboratory inspections with their corresponding non-conformities and observations, and the follow-up process conducted for the implementation of corrective actions.

If the laboratory has failed to comply with the requested corrections, the Inspector may request the National Directorate to immediately suspend the authorization for the Entity, or in duly justified cases, it may provide a new deadline, both for the implementation of corrective actions, as well as to reply to the observations.

2.2 PROCEDURES RELATED TO THE SUSPENSION OF ANALYSIS AND SAMPLING ENTITIES

Item 1.9.4 of this Chapter, on the termination of authorization and the application of sanctions, describes the powers of the National Fisheries and Aquaculture Service to sanction the authorizations granted to an entity, if it fails to comply with the requirements outlined in this Manual.

The following situations may lead to the suspension of the granted authorization:

a) Non-compliance with the requirements for the authorization of the entity, set forth in this Manual.
b) Repetitive non-compliance with the requirements set forth in this Manual for the operation of the entities.
c) Unjustified non-compliance with the requirements set forth by the Service.
d) Analytical or procedure errors, or deviations from the standards that lead to incorrect analyses results.
e) Not participating in mandatory competence trials as set forth in Item 1 of this Chapter.
f) Obtain unsatisfactory results in the proficiency tests, organized by SERNAPESCA and on behalf of the laboratory.
g) Loss of trust granted by the Service, that puts the certification process for export fishery products at risk.

Only those authorizations to perform the duties which through the General Fisheries and Aquaculture Law, SERNAPESCA has been empowered to delegate to the analysis and sampling entities will be suspended.

The suspensions to entities will only be issued by the National Directorate of the Service. To apply them, the affected entity must know beforehand the details related to the cause.

When starting the suspension, the entity must be informed of the necessary procedure to override the measure. An exception to this requirement would be if the suspension of the authorization were final.

Suspensions will last at least one week. To override a suspension, the entity must send a report of corrective actions to the National Directorate of the Service with a copy to the Regional Directorate. If the information provided suits the requirements of the Service and provides the requested solutions and guarantees, the entity may request an on-site inspection to the corresponding Regional Directorate. After the visit, the Inspector will issue a written report to the National Directorate. The suspension will be overridden immediately based on the result of this evaluation, or it will continue until the implementation of the required corrective actions is confirmed on site.

2.3 PROCEDURES RELATED TO THE INSPECTION OF ANALYSIS AND SAMPLING ENTITIES BY THIRD PARTIES

It is the obligation of the laboratories authorized by SERNAPESCA to timely inform the Regional Directorate of their jurisdiction, of any inspections or audits to the duties or activities included in the scope of the authorization, when it is conducted by a third party, which at the same time is involved in the authorization granted.

For instance, audits conducted by the National Institute of Standardization (INN) and the Public Health Institute (ISP) must be informed, as well as those conducted by any Chilean or foreign entities, when the scope of the audit includes aspects related to SERNAPESCA.

2.4 ADMINISTRATIVE PROCEDURES FOR INTERNSHIPS IN THE CONTEXT OF THE LABORATORIES PROGRAM

The main purposes of this activity are to standardize the inspection procedure, support the laboratory inspection activity and expand the knowledge of the Inspectors in the different areas of analysis included in the laboratories authorized by SERNAPESCA.

2.4.1 TYPES OF INTERNSHIPS

a) Internship of an Inspector at a SERNAPESCA Verification Laboratory

Consists of training an SERNAPESCA laboratories Inspector in a specific area of analysis of a SERNAPESCA Verification Laboratory.

Once the need for training is detected, the Laboratories Program of the National Directorate will coordinate with the SERNAPESCA Verification Laboratory the dates, times and topics to be covered in the internship, in consultation with the Inspector.

Once the internship has been concluded, the Inspector must send the National Directorate of the Service a report describing the course of the activity, observations, and conclusions. If the internship is included in an induction program, this must be included within the corresponding final report.

b) Internship of an Inspector at a SERNAPESCA office that does not belong to its jurisdiction

Consists of the visit of a Laboratories Inspector to a SERNAPESCA office that does not belong to its jurisdiction, with the purpose of standardizing inspection procedures.

The laboratories Inspector's internship must consider the following aspects:

- Coordination of activities between the internship Inspector and the assigned Inspector, so as to center priorities on drilling down the areas of interest.
- The visiting Inspector and the Inspector in charge must hold a kickoff and a closing meeting, so as to analyze any differences detected during the inspection.
- The internship Inspector together with the assigned Inspector will visit the programmed laboratories. The visit will be announced to the laboratory in advance, via e-mail.
- The results from the joint inspection visit must be written in an inspection report (addressed to the laboratory with a copy to the National Directorate), indicating that the purpose of the visit was an internal internship of the Service. The laboratory must reply to the final report, in the same way, and term as with quarterly inspections. If there are serious non-conformities, actions may be taken in that respect, after discussing it with the National Directorate.
- The final paragraph of the model laboratory report published on the Intranet of the Service must state the same as regular reports, however, the following must be added: "This visit was carried out by Inspector (name of the Inspector in charge of the laboratory) accompanied by (name of the internship Inspector)."
- An internship report must be sent to the National Directorate, with the purpose of conducting an analysis of the results obtained.
- This report must be written by both Inspectors and will be comprised of: Name of the visited entity, checklist used for the inspection and suggestions.

2.5 OVERSIGHT PROCEDURE FOR THE INSPECTION OF FISHERY PRODUCTS SHIPMENTS

The supervisions of inspections of export fishery products destined to the Eurasian Economic Union and Brazil, will be conducted by SERNAPESCA Official Inspectors from the jurisdiction where the activity takes place, who will verify on site the compliment with procedures and technical-administrative requirements set by SERNAPESCA, with the frequency deemed necessary. For this, the Inspectors must use the On-site Oversight Checklist for the Inspection of Export Fishery Products Shipment Destined to the EEU and Brazil (Part III, Annexes, Chapter III).

CHAPTER II. SAMPLING PROCEDURES AND METHODS

1. ADMINISTRATIVE PROCEDURES FOR SAMPLING ENTITIES

1.1 PROCEDURES FOR CONDUCTING THE SAMPLING OF EXPORT FISHERY AND AQUACULTURE PRODUCTS

These include the procedures to be followed by the officials of SERNAPESCA and the staff from the entities authorized by the Service, to conduct the sampling and analysis for the Certification, Quality Assurance (QAP), Live Bivalve Mollusks Sanitation and Residue Control Programs for export fishery and aquaculture products.

1.1.1 PROCEDURES RELATED TO THE CERTIFICATION PROGRAM

1.1.1.1. SAMPLING PROCEDURES

Any producers, exporters and their representatives that need to certify a lot of fishery products must go to the SERNAPESCA office assigned to the place of storage of the product, in order to obtain a Sampling and Analysis Request for Export (Part III, Annexes, Chapter II) which must be completed by the interested party, indicating, among other details, the registry number of the processing plant as per the list of companies under SERNAPESCA's Sanitary Control Programs, the name of the entity and the sampler that will conduct the sampling process, as well as the analyses to be conducted according to the destination market stated in the request. It must be based on what is set forth in Section III, Chapter IV, accordingly.

It must be noticed that when filing the request it must be declared if the product to be exported was produced with imported raw material, and the original fishery products entry request form (SIPP) associated with the product must be attached (Part III, Annexes, Chapter II).

Once the Sampling and Analysis Request for Export form is completed, it must be sent to the SERNAPESCA office corresponding to the place of storage of the product. The Service's official must verify all the information provided in the form, considering the following:

- Producer: It must be verified that the name of the producer corresponds to the Registered Name of the company. Fantasy names are not accepted. The processing plant's registry number must also be verified as per the List of Companies under SERNAPESCA's Sanitary Control Programs. Otherwise, a copy of the Resolution from the Undersecretariat of Fisheries authorizing the company for processing fishery resources must be attached.
- Place of storage: It must be confirmed that the place of storage of the product is under the Sanitary Control of SERNAPESCA, in whose case, the validity of the results report associated to the SMAE will have an equivalent validity as of the life of the product. Otherwise, the results report will be valid for 30 days from the date of its issuance.
- Category: The classification of the production facility must be confirmed in the List of Companies under SERNAPESCA's Sanitary Control Programs since the sampling plan to be applied to the product to be exported depends on it. If the facility is not categorized, the category "D" sampling plan will be applied only once and on an exceptional basis, while the categorization process is being conducted.
- Country of destination: If the product is destined to markets with specific requirements, included in Section III, Chapter IV, it must be confirmed that the processing facility is

authorized to export to those markets in the List of Companies under SERNAPESCA's Sanitary Control Programs.

- Batch Identification: The species must be clearly indicated, without any abbreviations, along with its full commercial and scientific name, the type of processing and the presentation of the product.
- Production Date: It must be verified that the number of boxes (secondary units) and the number of average units in them (primary units) is indicated in association to their production date since the size of the sample is determined for each production date. In the case of factory ships, the date of the corresponding tide is accepted as the production date.
- Expiration date or shelf-life: It must be verified that the producer has declared the expiration date or shelf-life of the products, according to their type of production and presentation.
- Sampling Entity: Both the entity and the sampler must be part of the List of Sampling Entities and the List of EFP Samplers and/or the List of BMSP Samplers of SERNAPESCA, accordingly, to conduct sampling tasks of export fishery products.
- Analysis Entity: The laboratory must be authorized to conduct the corresponding analyses and must be part of the List of Analysis Entities.
- Requested Analyses: The interested party must indicate the type of analysis being requested, according to the destination market or type of product. In the case of requiring additional analyses, requested through a letter of credit, these must be indicated as "Others" in item D of the Sampling and Analysis Request for Export (Part III Annexes, Chapter II).

The official of the Service, based on the type of product, category of the establishment and the country or market of destination of the product, must determine the number of samples to be extracted by production key, as indicated in Section III, Chapter IV. Also, once the information provided by the interested party is verified, the official of the Service must assign a number to the form, sign it and stamp it with SERNAPESCA's seal (Annex 1). The number assigned to the Sampling and Analysis Request for Export must be a correlative number, duly registered.

If the country of destination of the product is not specified, the official of SERNAPESCA must indicate in item "E - Remarks," the regulated markets to which the establishment is not authorized to export.

If the requestor is different from the producer, the official of SERNAPESCA must request the presentation of the original version of the tax document, as a proof of the processing fee or purchase of the product, before delivering the Sampling and Analysis Request for Export. The type of tax document and its identification number must be indicated in Item "E - Remarks" of the original version of this request. The copy of the tax document must be filed together with a copy of the Sampling and Analysis Request for Export.

The original version of the Sampling and Analysis Request for Export, signed and stamped by the official of SERNAPESCA, must be collected by the sampler responsible for conducting the sampling, indicating the day, time and place in which it will take place. The latter must be indicated with at least 24 hours in advance.

When due to force majeure, the responsible sampler cannot collect the Sampling and Analysis Request for Export, this can be done by the interested party or by the customs agency, only if the sampling is conducted by a sampler from the region. Before conducting the sampling, the sampler must inform SERNAPESCA, with at least 24 hours in advance, the date, time and place where it will be conducted.

If the sampler is registered to work in another region of the country, it must go to and identify himself in the office of SERNAPESCA, when collecting the Sampling and Analysis Request for Export.

The sampler must go to the place where the sampling will be conducted and carry out the procedure by extracting the number of samples described in the Sampling and Analysis Request for Export, according to the specifications and procedures set forth in Item 2 of this Chapter.

The sampling entity will be responsible for keeping the samples in optimum conditions, as set forth in Item 2 of this Chapter, from the moment in which they are taken to their arrival at the analysis laboratory.

If the sampling does not take place, the sampler must send a written notice to SERNAPESCA, indicating the reasons for its suspension. Depending on the causes for its suspension and of the date in which it will possibly be conducted, SERNAPESCA will determine, as appropriate, voiding the Sampling and Analysis Request for Export. In this case, the number assigned to this request must also be voided.

Once the sampling has taken place, the sampler must issue a Sampling Report, which must be signed by the person responsible for its execution.

1.1.1.2. SPECIAL CONSIDERATIONS IN THE SAMPLING PROCESS

a) Sampling of lots of 500 kg or less

In the case of small shipments comprised of production lots of 500 kg. or less, the sampling plans and microbiological determinations described in Section III, Chapter IV, Item 1, will be applied, considering "n" equal to 5, regardless of the category of the processing establishment.

b) Bivalve Mollusks, Gastropods, Echinoderms, and Tunicates

In the case of species susceptible to marine toxins, the number of samples to be extracted for conducting the biotoxins analysis must also be indicated in the Sampling and Analysis Request for Export, as per Section III, Chapter IV, Item 1.

When issuing the Sampling and Analysis Request for Export, the interested party must present the SMAE with the Sworn Declaration of Origin (Part III, Annexes, Chapter II) and the supporting tax documentation from the purchase of the raw material at origin to the person responsible for presenting the SMAE. (Tax document of origin, purchase or sale at the place of extraction, and the transfer tax document that prove traceability). The company must file a copy of the Sworn Declaration of Origin, as well as the tax documentation indicating the supporting SMAE number. These documents will be filed and must be available at the plant for their review when inspecting the Categorization of the facility.

In the case of exporting bivalve mollusks, gastropods, echinoderms, and tunicates to the European Union, it must be verified that the resources come from areas that are part of the List of Extraction Areas of the BMSP.

c) Special Sampling Plan for Products Manufactured under the Same Key

This sampling plan applies to those products of the same key or work day, produced in different presentations or packed in different formats, either under the request of the buyer or due to a specific situation of the product, without being subject to any treatment after their packing.

A proportional sampling plan must be applied according to the following procedure:

- Establish the number of samples from the lot according to the category of the processing establishment.
- Establish the total weight per type of packaging or presentation.
- Establish the weight percentage, per type of packaging or presentation, in relation to the weight of the entire lot.
- Determine the number of samples to be extracted for each format or presentation according to the previously obtained percentages.
- The number obtained will correspond to the number of boxes to be selected by type of packaging or presentation.
- Extract a unit of each selected box.
- The sample will be comprised of at least one unit of each format or presentation.
- Samples must be extracted according to the same procedure, for microbiological and physical-chemical analyses.

The acceptance number will be applied considering all the units analyzed (n) regardless of their format or presentation.

d) Request for Sampling and Analysis for Export in the case of repacking the end product

When the fishery product to be exported needs to be re-packed after the sampling, the interested party must inform the Service. If deemed necessary, SERNAPESCA will oversee the operation and will request, when applicable, a new sampling. This new sampling will be required every time that the re-packing involves handling and when there is a risk of contaminating the product.

Those processing plants that have included the "re-packing" operational procedure in their QAP may conduct the re-packing operation according to what is set forth in Item 1.1.3 c of this Chapter.

e) Re-packing Products with Primary Packaging

The re-packing of fishery products, after the sampling of the product, is authorized when these are packed in the original packaging and their handling does not involve contamination. This operation must be informed to SERNAPESCA by the interested party so as to show proof for certification purposes.

For this, the interested party must request the re-packing, presenting the original version of the Request for Sampling and Analysis for Export at the SERNAPESCA office where the product is located to conduct the sampling and the corresponding report. The staff of the Foreign Trade Sub-Directorate of the region must stamp in the sampling report and in the Request for Sampling and Analysis for Export "Authorized Re-packing" with their initials and the seal of SERNAPESCA.

The re-packing process must be supervised by an authorized sampler, who will issue a "packed products re-packing report (secondary packaging)," which describes the change of format and the conditions in which the procedure took place. This report must be presented with the original sampling report, together will all the information required for certification.

f) Re-packing Bulk-packed Products or without Primary Packaging

When it is necessary to re-pack a fishery product that was originally sampled, and that is bulk-packed or does not have a primary packaging, the interested party must request a prior authorization to SERNAPESCA, presenting a new Request for Sampling and Analysis for Export.

For this, the official of SERNAPESCA must write the number of samples to be extracted in the new Request for Sampling and Analysis for Export, expressly stating in the initial Request for Sampling and Analysis for Export "Invalid request due to re-packing with handling", leaving a copy of it and the corresponding sampling and analysis report at SERNAPESCA. The re-packing operation must be supervised by an authorized sampler, and the product must be re-sampled according to the sampling plans set forth in Section III, Chapter IV, with the purpose of evaluating their sanitary condition after handling.

The authorized sampler must issue a complete sampling report (Item 2.16) that expressly states the new number of primary units and net kilograms obtained after re-packing, also attaching all the information required for certification.

g) Inspection Procedure for Reentering Fishery Products

This procedure is applicable to the inspection of fishery products reentered to the country, for which it is necessary to verify the consistency of the information and the sanitary condition of the product, which requires to create a *packing list* and to conduct an aerobic mesophilic and organoleptic microorganisms count analysis for refrigerated or frozen products, based on what is described in Section V of this Manual.

For this, the Inspection must be conducted by Export Fishery Products (EFP) samplers authorized by SERNAPESCA and according to the sampling regulations in place. The samplers must have a copy of the Fishery Products Entry Request (SIPP) and the Notification of Shipment (NEPPEX) with which the export of the product to be inspected was originally authorized.

The minimum required information to be included in the inspection report is described in Item 2.1.7.

The samplers must have all the necessary supplies and attire for conducting the inspection, that is, a thermometer, 70% alcohol, thermal clothing, sterile gloves and safety shoes.

h) Inspection and Sampling Procedure for Export Fishery and Aquaculture Products Shipments with Destination to the Eurasian Economic Union (EEU) and Brazil

This procedure is applicable for the inspection of export fishery and aquaculture products with destination to the Eurasian Economic Union (EEU) and frozen salmon products in any presentation to Brazil, for which it will be necessary to verify and clearly identify all the products to be shipped, also only for salmonids, it must be verified that the products do not have any injuries attributable to infectious diseases and that they are at a temperature of ≤ -18 C°.

For this, the Inspection must be conducted by Export Fishery Products (EFP) samplers authorized by SERNAPESCA according to the sampling regulations in place. In addition to complying with the indications described in Chapter II, Item 2.1.3.10 of this Section and in Section III, Chapter III and notifying the regional office at least 24 hours before the sampling. After the consolidation, whether it is unique or partial, and if it complies with the requirements for the export of products destined to Brazil and the EEU, the authorized sampler will seal the load and issue the inspection and sampling report.

The minimum required information to be included in the inspection and sampling report is described in Chapter II, Item 2.1.8 of this Manual. The report must always include the full name and signature of the sampler.

The samplers must have all the necessary supplies and attire for conducting the inspection, that is, a thermometer, 70% alcohol, thermal clothing, sterile gloves and safety shoes.

i) Compliance with the Requirements Established

The Export Fishery Products samplers must comply with all the requirements established. If there are any non-compliances with these requirements, sanctions will be applied, which go from a temporary to an indefinite suspension from SERNAPESCA's system.

1.1.2 SENSORY EVALUATION PROCEDURES

The producer, exporter or its representative, that needs to certify a lot of fishery products must go to the SERNAPESCA office of the place of storage of the product in a chilled-refrigerated condition, in order to deliver the Notification of Shipment of Export Fishery Products, after completing items A, B and C, as described in Section III of this Manual. The SERNAPESCA official must verify all the information, indicate the sampling n value and location, to then sign and stamp the document with the Service's stamp.

The official sampler of Export Fishery Products must conduct the sensory evaluation of those products that are exported in a chilled-refrigerated condition, and for this, it must have the original version of the Notification of Shipment of Export Fishery Products.

It must verify that the information contained in the Notification of Shipment of Export Fishery Products corresponds to that of the product provided for sensory evaluation, as set forth in Item 3 of this Chapter. Then, it must extract the number of required boxes, as per the instructions provided in Item 3 of this Chapter, and examine the product inside each one of them, considering the characteristics described in the same standard.
Once the procedure has taken place, the authorized sampler must issue an original of a Record of Sensory Evaluation and two copies, which must contain the information described in Item 3.34 of this Chapter. The original must be delivered to the interested party, and the copies must be delivered to SERNAPESCA and the Sampling Entity, respectively.

If the processing of official certificates takes place in a region different from the place in which the sensory evaluation was conducted, the Sensory Evaluation Record must be reviewed by the official of the SERNAPESCA office of the location of the evaluation, as per the procedure described in Item 1.1.1.

a) Internal Control of the Sensory Evaluation Record

This procedure will only be needed if the certification that takes place in a different region where the sensory evaluation took place.

The internal control of the Sensory Evaluation Record must be done in the office of SERNAPESCA of the place in which the procedure takes place, and its purpose is to testify that the Sampling Entity is operating normally and to verify that what is stated by the Inspector in the record agrees with what is stated in the Notification of Shipment of Export Fishery Products and with what was informed and verified on site by the SERNAPESCA official, if the sensory evaluation has been inspected.

For this, the interested party must present the Notification of Shipment of Export Fishery Products and the Sensory Evaluation Record at the SERNAPESCA office under which jurisdiction the evaluation took place.

If the sampling entity is operating normally, the staff of the Foreign Trade Sub-Directorate will verify what was stated in the Sensory Evaluation Record in terms of the place where it took place, the description of the inspected product, production dates of the product, number of samples evaluated, presentation of the product, among others, and how it matches the information included in the Notification of Shipment of Export Fishery Products.

Once the information has been verified, the document will be stamped with the SERNAPESCA seal, dated and signed with the initials of the official responsible for the procedure, on the front side of the first page of the Sensory Evaluation Record. Otherwise, if the information is not consistent or if there are any irregularities, the internal control procedure will not take place.

If the results of the sensory evaluation are out of range, as set forth in Section III, Chapter IV, Item 1, the internal control procedure must equally take place, highlighting any unfavorable results and informing this to the interested party.

1.1.3 PROCEDURES RELATIVE TO THE QUALITY ASSURANCE PROGRAM (QAP)

The sampling for the QAP verification must be performed by samplers authorized by SERNAPESCA

a) Sampling for Verification of SERNAPESCA

Before conducting the sampling, the processing plant must collect the QAP Samples Delivery Form (SDF – *FEM*) from the office of SERNAPESCA of its jurisdiction, where the corresponding pages collected will be recorded. Moreover, the sampler must inform the SERNAPESCA office, with at least 24 hours in advance, the time and date when the form will be collected.

The sampler must go to the place where the sampling will be conducted and carry out the procedure by extracting the number of samples described in the QAP, according to the specifications and procedures set forth in Item 2 of this Chapter. Only one product will be sampled for each QAP Verification Samples Delivery Form. The SERNAPESCA official will select the samples to be extracted, and their delivery is the responsibility of the sampling entity.

The samples must be sent to the SERNAPESCA Verification Laboratory, together with the QAP Verification Samples Delivery Form (Part III, Annexes, Chapter II), in original, validated with the name and signed with the initials of the SERNAPESCA Inspector present during the sampling. The other two copies will be held by SERNAPESCA, and the processing plant, respectively. Whenever possible, the form must be sent inside of the box carrying the samples. This box must be sealed with SERNAPESCA's adhesive tape.

Once the sampling has been conducted, the sampler must issue a report to the interested party, as set forth in item 2.16, which must be filed by the processing establishment.

b) Sampling for Verification at the Service Laboratory

Before conducting the sampling, the processing plant must collect the QAP Samples Delivery Form (SDF - *FEM*) from the office of SERNAPESCA of its jurisdiction, where the corresponding pages will be recorded. Moreover, the sampler must inform the SERNAPESCA office, with at least 24 hours in advance, the time and date when the form will be collected.

The sampler must go to the place where the sampling will be conducted and carry out the procedure by extracting the number of samples described in the QAP, according to the

specifications and procedures set forth in Chapter II, Item 2. Only one product will be sampled for each QAP Verification Samples Delivery Form. The authorized sampler will select the samples to be extracted, and their delivery is the responsibility of the sampling entity.

The samples must be sent to the authorized Service Laboratory, together with the original QAP Verification Samples Delivery Form (Part III, Annexes, Chapter II). If a SERNAPESCA Inspector is present during the sampling, it must validate the SDF with its name and sign with its initials. The other two copies will be held by SERNAPESCA, and the processing plant, respectively. The form must be sent together with the box carrying the samples. This box must be sealed with the sampling entity's adhesive tape.

Once the sampling has been conducted, the sampler must issue a report to the interested party, as outlined in item 2.16, which must be filed by the production facility.

c) Sampling Procedure in Case of Repacking Products Processed at Establishments with a Validated Quality Assurance Program

Repacking products manufactured in establishments with a validated QAP may only be authorized if this procedure is described on the plant's QAP, which must be verified by the SERNAPESCA office of the region of origin of the product, prior to the issuance of the Authorization at Origin for Sanitary Certification (AOCS).

If on the contrary, this risk is not considered in the QAP or if the repacking is done by an exporter different from the manufacturer, beyond the control of the plant's Quality Assurance Program, the process described in Item 1.1.1.2 of this Chapter should take place.

1.1.4 PROCEDURES RELATIVE TO THE CONTROL OF PHARMACEUTICAL PRODUCTS RESIDUES, PROHIBITED SUBSTANCES, UNAUTHORIZED SUBSTANCES AND CONTAMINANTS IN AQUACULTURE

The sampling of fish in farms, with the purpose of carrying out residues and contaminants analyses, to support the Declaration of Guarantee, must take place as described in Item 2 of this Chapter, and Section 1, Chapter II.

To conduct the analyses, the samples must be sent to the laboratories authorized by SERNAPESCA, included in the List of Analysis Entities, under proper conditions, as set forth in Item 2 of this Chapter.

a) Sampling and Analysis Procedures for the Verification of SERNAPESCA (FAR)

For SERNAPESCA's Verification sampling, the samples must be obtained as described in Item 2 of this Chapter and Section I, Chapter II. Afterward, they must be sent under proper isolation and temperature conditions directly to the SERNAPESCA Verification Laboratory of the Program, as described in the List of Analysis Entities. The samples must be sent together with the QAP Verification Samples Delivery Form (FEM-QAP) (Part III Annexes, Chapter II). The company will be responsible for sending the samples, and it must inform their delivery to the SERNAPESCA Verification Laboratory, via phone or fax.

b) Drugs Residues and Contaminants Analysis Procedures in Authorized Laboratories

The authorized laboratory must verify the information accompanying the samples so that it matches the samples received for analysis. Similarly, it must make sure that the samples are in

proper conditions to start the corresponding analyses. If the samples have not been received in proper conditions for their analysis, the entity must inform this situation to the interested party, with a copy to the SERNAPESCA National Directorate, to carry out a new sampling if deemed necessary.

It must conduct the procedures described, according to the analysis methodologies authorized by SERNAPESCA, as per the requirements set forth in Chapter III, Item 4.
Once the analyses have been conducted, the entity must issue an Analysis Report providing the information outlined in Chapter III, Item 1.5.

If the results of the analysis are unfavorable or if they fall outside the parameters set forth by SERNAPESCA in terms of the product, for samples of the Residues Official Verifications Program, samples of the Prohibited Substances, Contaminants and Unauthorized Substances Control Program or for samples coming from the Certification Program, the National Directorate of the Service must be informed of the situation immediately and no later than 12 hours, as described in Chapter III, Item 1.5. The National Directorate will inform the region of origin of the producer and the certifying regions.

If the results of the analyses are unfavorable or if they fall outside the parameters established by SERNAPESCA, for pre-harvest samples of the Residue Control Program, these must be informed to the National Directorate of the Service within 30 days and the laboratory's monthly activity statistics template may be attached, as set forth in Chapter III, Item 1.6.

1.1.5 PROCEDURES RELATIVE TO THE BIVALVE MOLLUSKS SANITATION PROGRAM (BMSP)

a) Sampling Procedures for Classification and Monitoring

Taking and delivering samples, as well as coastline evaluations, must be conducted by authorized samplers that have approved the Official Course for BMSP Samplers (according to the program described in Chapter I, and that are registered in the BMSP Samplers List).

Before conducting samplings to classify or monitor areas, the BMSP sampler must go to the SERNAPESCA office controlling the areas and will deliver the following information on a weekly basis:

- Monitoring programs to be applied during the week, so as to collect the required BMSP Sampling and Analysis forms stamped by the SERNAPESCA Inspector.
- Sampling forms and the SERNAPESCA copy, used the previous week.
- Reports of the samplings conducted the previous week, describing the conditions at the moment of the sampling (weather, notes on the activities taking place in the area or in the coastline, or any other adverse contamination conditions).

Similarly, a summary report including the information described in the following table must be delivered:

Table: *Record of the forms used and samplings dates*

Sampling Date:	Area: Name and Number	Producer	Analysis to be conducted

This summary must have a unique and correlative identification that will be assigned by the sampling entity, and a copy of this document must be filed. The purpose of this procedure is to have

a record of the forms used and the sampling dates, so as to conduct the follow-up of the analyses carried out and to control the response time of the laboratories.

Afterward, the procedure described in Item 2 of this Chapter must be conducted on site, extracting the number of samples specified in the classification or monitoring program of the area, in accordance with the procedures set forth in Section I, Chapter I.

It should be noted that the samples must always be extracted from the sampling stations assigned for each type of analysis. If this is not possible, the sampler must inform the situation to the corresponding SERNAPESCA office.

Each sample must be accompanied by the original BMSP Sampling and Analysis Form (Part 3, Annexes, Chapter II), which must be completed with all the required information, indicating if the sample is sent for microbiological, chemical, toxicological or phytoplankton analysis. Each time the area is visited to conduct the sampling, the authorized sampler must measure the temperature of the water and indicate this measurement in the BMSP Sampling and Analysis Form corresponding to the microbiological sample. If there is no microbiological sampling at that time, the temperature will be informed in the form for the phytoplankton sample.

The original form will accompany the samples to be delivered to the analysis laboratory, and the interested party must keep a copy.

Based on the analysis required, the copies must be sent to authorized laboratories, accordingly, included in the List of Analysis Entities.

The samples must be sent to the laboratories according to the instructions provided in Item 2 of this Chapter and under the sole responsibility of the sampler, who must ensure that such samples are sent to the analysis laboratory in a timely manner.

1.2 COMPLIANCE WITH THE REQUIREMENTS ESTABLISHED FOR CONDUCTING THE SAMPLING AND ANALYSIS OF EXPORT FISHERY AND AQUACULTURE PRODUCTS

The samplers and analysis entities must comply with all the requirements set forth. If there are any non-compliances with these requirements, either from the analysis laboratories or the samplers, sanctions will be applied, which go from a temporary to an indefinite suspension from SERNAPESCA's system.

These non-compliances subject to sanctions for samplers are the following, among others:

- Delays in sending the samples to the analysis laboratory.
- Non-compliance with the monitoring program.
- Attending a sampling without the necessary equipment.
- Sending samples to unauthorized laboratories.

These non-compliances subject to sanctions for analysis entities are the following, among others:

- Delays in sending the results of the analyses.
- Non-compliances in the unfavorable results report.

2. EXPORT FISHERY PRODUCTS SAMPLING METHODS

Sampling procedures are included to obtain a representative sample of food and move the sampling units to the laboratory in identical conditions to those it had during the sampling. The samples must be obtained by authorized and properly trained personnel.

The procedures described in this standard apply to the sampling procedure for the Certification, Quality Assurance (QAP), and Live Bivalve Mollusks Sanitation (BMSP) Programs. Fish meal and fish oil sampling are also included.

2.1 CLOTHING, PERSONAL PROTECTIVE EQUIPMENT, AND LABORATORY SUPPLIES TO BE USED BY THE SAMPLER

2.1.1 CLOTHING AND PERSONAL PROTECTIVE EQUIPMENT TO BE WORN BY THE SAMPLER

The sampler authorized by SERNAPESCA must wear proper clothing and personal protective equipment which must include at least the following:

- White apron.
- Disposable gloves, as needed.
- Hairnet or hat.
- Face mask, as needed.

2.1.2 SUPPLIES TO BE USED BY THE SAMPLER

a) Types of Laboratory Supplies

Containers for samples: Clean, dry, airtight and sterile containers must be used, such as wide mouth glass jars, cans or plastic bottles, stainless steel containers or disposable plastic bags, with the proper capacity to hold the sample to be extracted, considering at least 250 g per sample unit. Reusable containers must be of a proper quality to allow for sterilization. Screw caps must be used when using containers with caps. Their material must be insoluble and non-absorbent. Rubber, plastic or cork stoppers may be used, to the extent that they are lined with inert material such as aluminum foil or plastic, before being placed in the sampling container. Disposable plastic bags must be firmly sealed after filling them so as to avoid any leaks or spills during their handling.

In the particular case of taking samples to analyze PAH, plastics such as propylene, PFTE or similar must be avoided, only aluminum, stainless steel or glass containers may be used, protecting the sample from light. Before their use, containers must be washed with high-purity acetone or hexane so as to minimize the risk of contamination.

For the sampling of Dioxins and PCBs, the containers must be requested to the laboratory conducting the analyses, who will carry out the cleaning process of them according to international protocol, in order to avoid contamination of the samples. The material with which the sample will be handled must be properly cleaned with alcohol prior to the sampling.

- Sampling instruments: Commonly-used tools, punches, probes, drills, spoons, scoops, shakers, pipettes, cotton balls, as required. Probe or spears for fish meal. Probe for extracting fish oil samples. All instruments must be properly sterilized.
- Instruments to open food packages: Scissors, knives (as needed), properly sterilized.
- Labels: Adhesive paper labels of a proper size where all the relevant data of the sample can be included (Item 2.14).

- Sterilization equipment: Autoclave, oven capable of reaching a temperature of 170 C°, gas sterilization chamber. A small autoclave (between 20 to 30 L), a vapor chamber or gas or alcohol burner may be used as portable equipment, as required.
- Insulated container: Plastic foam box or isothermal box for transporting frozen or refrigerated samples.
- Gel packs or dry ice: To maintain the temperature of refrigerated or frozen product's samples.
- Sterilizing agents: 70% alcohol, ethyl or isopropyl, sodium hypochlorite solution with at least 100 ppm of free chlorine.
- Thermometer: Suitable for the type of products to be sampled, ideally metallic with a temperature range between -35 and 100 C°, with grade intervals not exceeding 10 C°; their accuracy must be periodically checked against a duly calibrated standard thermometer.
- *Global Position System (GPS):* All samplers that are part of the Live Bivalve Mollusks Sanitation Program must have one.

All measurement devices used in the sampling process must be properly calibrated and verified.

The sampler is responsible for taking all the necessary supplies and equipment to conduct the sampling to the corresponding location. Otherwise, the sampling will be suspended.

b) Sterilization of Supplies

- All containers intended to hold the samples and any tools that will come in contact with the food must be sterilized. As a general rule, the supplies and tools must be previously sterilized at the laboratory with some of the following methods:
  - Autoclave (121 C° for 15 minutes). This procedure is recommended for containers and supplies that may suffer damages with dry heat (for instance, rubber bands).
  - Exposure to dry heat in an oven at 170 C° for at least 90 minutes.
  - Ethylene oxide with carbon dioxide. This procedure can be used with plastic containers. However, it requires certain precautions.
  - Ionizing radiation, for instance, for plastic bags.

- There must be enough pre-sterilized supplies and tools (punches, spoons, etc.) brought from the laboratory to obtain the required number of samples, or they could be cleaned and sterilized in the place where the samples are taken through the following methods:
  - Portable autoclave (121 C° for 15 minutes).
  - Steam exposure at 100 C° for one hour.
  - Heating in a portable oven at 170 C° for one hour.
  - With a Bunsen burner or a gas burner.
  - Immersion in ethyl alcohol (70% v/v) and then passing through a flame to eliminate alcohol.
  - By immersion, at least during 30 seconds, in a solution of sodium hypochlorite with a minimum of 100 ppm of free chlorine or another halogen with equivalent bactericidal power; it must be rinsed with sterile water and dried with a sterile cloth before its use.

- The sterilized material in the laboratory must be labeled with the sterilization date.

2.1.3 COLLECTING SAMPLES

2.1.3.1 QAP CERTIFICATION AND VERIFICATION SAMPLING

Before conducting the sampling of an export fishery product, the official sampler must make sure that it has the necessary documentation and equipment to carry out the task, according to the

Food Safety and Certification Manual. He must also make sure that the lot to be sampled is completely present at the sampling location. If the lot to be sampled is incomplete, the sampler must suspend the procedure and immediately inform SERNAPESCA. If SERNAPESCA is not informed when the lot is incomplete or if it does not correspond to what is described in the Request for Sampling and Analysis for Export it will be subject to sanctions.

a) Number of Sampling Units:

For Certification sampling, the number of units described in the Request for Sampling and Analysis for Export and in Section III, Chapter IV, Item 1, must be extracted.

For QAP verification sampling, the number of sampling units described in the plant's QAP must be extracted to carry out all the corresponding analyses for the presentation of the product. Adding samples of different products for the same analysis is not allowed.

b) Obtaining the Sample:

- The sampler must adopt all the necessary measures to prevent the contamination of the batch of food or of the sampling units, as well as bacterial growth or death in the samples, during their collection and transportation to the analysis entity.
- The samples must be taken in the place of storage of the product, randomly for the entire lot to be sampled. For this, Chilean Standard NCh 43 "Random Collection of Samples" must be used. The quantity of the sample to be extracted must be enough to conduct all the analyses corresponding to the type of product and to keep a reserve sample in case of an accident or for further research.
- In the case of products with primary and secondary packaging, random sampling must be understood as that only applied to the secondary packaging, so as to not conduct unnecessary handling of the product.
- If the number of secondary packaging is lower than "n," more than one primary packaging may be extracted from each one, until completing the size of the sample. Similarly, if a primary packaging is too small to conduct the required analyses, the number of primary packaging with the necessary amount of product to conduct the analyses must be extracted from each secondary packaging.
- In the case of block frozen products, if the conditions of the sampling location do not comply with minimum sanitary requirements during the sampling, it is recommended to extract "n" blocks of product to send to the laboratory.
- The sampled units must be identified with packing tape or the label of the sampling entity.
- The samples must be sent to the laboratories in their original and closed packaging, whenever possible.
- If the product is packed in large primary packaging that cannot be transported to the laboratory or that have a higher amount of product to that required for the analysis, the fraction of food corresponding to the sample may be transferred to a sterile container, under aseptic conditions. Superficial contamination must be eliminated from the primary packaging in the areas where the product is to be extracted, washing it to remove dirt or with a sponge with alcohol, and then rubbing it with 70% alcohol. The package must be opened with a sterile cutting instrument such as scissors or a knife, as appropriate. A different instrument must be used for each package, or it must be sterilized before each use, so as to avoid cross-contamination. For air-tight containers, these must be sent unopened to the laboratory.
- If the food is in bulk, the sample must be obtained from several points of the container, unless there is evidence that the product is completely homogeneous or that there is information that shows the convenience of extracting the sample in a specific area.
- To obtain the sample, the most suitable instrument for the physical state of the food must be used; for instance, a saw, knife or punches for frozen products, pipettes for liquid products, scoops or spatulas for dried food, etc.

- The most representative sample must always be extracted, homogenizing the product if needed.
- In the case of fresh refrigerated or frozen products, the temperature in the highest temperature area of the storage room for the samples as well as of the sampled food must be recorded. This must be done by inserting the thermometer immediately after extracting the sample, sterilizing it before every use. If small sealed containers are going to be sent to the laboratory, the temperature of the food from an adjacent container in the same cardboard box or packaging must be recorded.
- For samples intended for chemicals and heavy metals analyses, the extracted sample must be enough to obtain the counter sample and conducting the requested analysis.

2.1.3.2 SAMPLING FOR THE BIVALVE MOLLUSKS SANITATION PROGRAM BMSP

The following is a description of the procedure to be used to conduct the sampling of bivalve mollusks, for the classification and monitoring of extraction areas that comprise the microbiological, chemical, toxicological analysis of the resource and of phytoplankton, based on their origin.

a) Sample Size:

- Microbiological: The sample will be comprised of a number of units that allows having at least 10 animals and 200 g of flesh.
- Chemical: The sample will be comprised of 200 g of flesh.
- Toxicological: A minimum of 12 units and 200 g of flesh per toxin to be analyzed is required (PSP, ASP, and lipophilic toxins).
- Phytoplankton: The size of the samples for phytoplankton analysis is described in item 2.1.3.2.c).

b) Obtaining the Microbiological, Chemical and Toxicological Sample:

- The sampler must adopt all the necessary measures to prevent the contamination of the batch of food or of the sampling units, as well as bacterial growth or death in the samples, during their collection and transportation to the analysis entity.
- The sampling must be conducted in the extraction area, whether it is a farm or a natural bank, collecting the required units randomly, and which must be alive, discarding damaged specimens. In the case of samples from farms, these must be collected by extracting the product from different depths.
- The sample comprised of specimens in their shell will be placed in isothermal boxes with a refrigerant (*gel pack* or its equivalent), so as to keep its temperature under control (0 to 10 C°) until the analysis takes place. Microbiological and toxicological analyses must not be conducted 24 hours after taking the sample, except those samples coming from the Magallanes Region, which consider 48 hours. The analyses of chemical samples (heavy metals and contaminants), must take place within 48 hours of taking the sample.
- The samples may be sent frozen only when expressly authorized by SERNAPESCA, or in the case of toxicological and/or chemical samples coming from the Region of Magallanes.
- The samples must be properly individualized and labeled so as to know the exact origin of the raw material and the reception date at the plant. The information included on the label must show at least the Sampling Form No., the number and name of the extraction area, the identification of the monitoring station, and the time and date of the sampling.
- A "*Waypoint*" must be marked on the sampler's GPS for each sample collection point, recording the exact time and location where the sample was collected. This information may be audited by SERNAPESCA Inspectors.

c) Collecting Samples for Phytoplankton:

A "*Waypoint*" must be marked on the sampler's GPS for each sample collection point, recording the exact time and location where the sample was collected. This information may be audited by SERNAPESCA Inspectors.

The following describes the procedures for collecting samples and conducting analyses for phytoplankton.

- Qualitative Sampling:

Samples must be collected with a phytoplankton net, which consists of a PVC or stainless steel ring in the mouth, a filtering part of fine mesh with an opening between 20 – 25 μ, a cod-end and a dead weight of more than one kilogram that can facilitate stressing the cable supporting the net, which will have a minimum length of 35 meters, and marked every meter.

Before submerging the net in the water, the depth of the point must be confirmed so as to avoid touching the bottom with the net and re-suspending sediments.

The samples will be collected at the sampling points or stations predefined in the classification and monitoring programs. The net must be submerged in two points in each sampling station. The distance between these two points depends on the hydrography of the area, but it must aim at 100 m.

In each one of the two points, the net must be sunk at a depth of 20 meters and then towed three times vertically, these will become a single sample (6 tows in a single sample). The samples must be collected with the vessel anchored, and when raising the net, the speed must be continuous and without pulling.

The qualitative sample collected must be placed in a plastic jar with a 500 ml capacity, filling the jar with 450 ml of sample (the sum of the 6 tows), filling it with formalin or lugol so as to reach a final concentration of 3% to 4%.

The sampling entities must implement net maintenance programs, considering a periodic calibration according to the frequency of use given to the net. If used on a monthly basis, it is recommended to conduct a quality check at least once a year.

The samples must be labeled with at least the following information:
- BMSP Sampling Form No.
- Number and name of the extraction area.
- Type of sample (qualitative or quantitative).
- Monitoring station.
- Time and date of the sampling.

At the end of the process, each station must wash the net to avoid the contamination of the following samples. For this, it is recommended to have a container on board with fresh water for rinsing purposes. After the sampling is completed, the net must be washed inland with fresh water to eliminate any traces of seawater.

The samples must be carefully packed to avoid spills or breakage during transportation. It is recommended to close them tightly and then placing them in a *Styrofoam box or cooler*.

- Quantitative Sampling:

The hose sampling procedure is recommended to collect an integrated sample from the water column. For this, a regular hose of approximately 2.5 cm in diameter and between 18 to 20 meters in length is used to integrate the first 15 meters of the water column. The end that is sunk must have a dead weight of 5-7 kilograms tied to it, and which allows keeping the hose straight when sinking it, and a rope tied to the other end to raise it once the sample is collected.

The vessel must be stationary when collecting the samples. The hose must be firmly attached to the vessel, and it must be marked every 5 meters to know the depth at which it is being sunk. The hose must be sunk at a steady speed until reaching 15 meters. This is when the hose must be raised to then break its upper end to create a vacuum, and pulling the rope at the other end to start raising it.

Once it is on the deck, the sample must be placed in a plastic container to then homogenize it and collect a sub-sample in a 100 ml plastic jar. Afterward, the fixative must be added to the sample, in this case, it is 7 to 10 drops of lugol.

The information protocol corresponding to the sample is the same as for qualitative samples, except for the fact that the depth at which the sample was collected must be indicated in case it was less than 15 meters.

The container where the sample is placed must have enough room to homogenize the sample.

The samples must be carefully packed to avoid spills or breakage during transportation. It is recommended to close them tightly and then placing them in a *Styrofoam box or cooler*.

2.1.3.3 FISH MEAL SAMPLING:

The following describes the procedure to be used when conducting fish meal sampling. The following is the specific terminology for this type of product:

a) Supplies and Equipment:
The sampling entity must have the necessary supplies to conduct the task. These include the following:

- Probes.
- 1.2 m, 1.5 m, and 5 m sampling spears.
- Spoons or scoops with a minimum capacity of 25 g.
- Burners.
- Sterile polyethylene or first use bags.
- Disinfecting agents and cotton.
- Folding ladder.
- Duct tape or other with similar characteristics.

When handling polyethylene bags, it must be guaranteed that its sterility will not be lost during its storage and further use.
The elements to be used must be disinfected before collecting the samples and between each lot to be sampled. Similarly, the area where the sacks from which the increments to be extracted are located must be thoroughly cleaned and disinfected. In addition, the puncture left after introducing the probe must be closed.

Samplers must wear clean and proper clothing and personal protective equipment, including at least:

- Safety shoes or boots.
- Helmet.
- Face mask.
- Gloves.
- Coverall.

b) Sampling Procedures

The following samplings procedures for each one of these product's presentations, consider the collection of a single representative sample, comprised of the addition of increments extracted from a lot. That is to say, the sampling procedure consists of collecting one single sample per lot, through the collection of a certain number of increments per ton.

Exceptionally, in Section III, Chapter I, Item 2, n ≠1 could be specified, according to the requirements of the market, for instance for the European Union and Chinese markets where n=5 is defined. In these cases, the sampling collection procedure for the different presentations of fish meal must also consider a certain number of increments per ton. However, these must be placed in as many separate bags as indicated by the sampling n and they should be sent in the same way to the laboratory. The above, always considering the same total minimum volume of the sample, described in this standard and based on its presentation.

The probe or spear must always be passed through a flame after withdrawing it from a bag during the sampling process.

In the specific case of the samplings conducted in meal packed in sacks, the company is responsible for avoiding that the product becomes exposed or that it leaks through the area where the sample is collected, so as to avoid the contamination of the product.

Meal packed in sacks: The lot must be sorted in such a way that at least 60% of the sacks are available for sampling. It must also comply with the following requirements:

- It must be arranged in a safe and stable way for the staff in charge of conducting the sampling.
- The lots must be surrounded by a clear area of at least 70 cm in its entire perimeter.

Meal packed in 50 kg sacks: Two increments per ton must be collected from each inspection lot, with a probe of approximately 45 cm of length, as follows:

- The increments must be collected randomly from different sacks, from the sides of the lot, following an imaginary "V"-shaped line, and an inverted "V"-shape to collect samples of the opposite side. The increments must be collected from the top, mid and lower areas of each side of the lot.
- At least 1,500 g of representative samples must be collected from each lot.

Meals packed in 1-ton sacks (*jumbo bags* or *maxi bags*): 20 % of the sacks that comprise the inspection lot must be sampled, selecting 1 *jumbo bag* out of every fifth, keeping a continuous interval between the selected sacks.
The procedure must take place by inserting the sampling spear through the upper opening of the sack, collecting at least one increment per sack.
At least 2,000 g of representative samples must be collected from each lot.
The increments must be poured in a moisture tight, sterile (or first use) and properly identified container, until completing the entire representative sample. Once in the laboratory, the sample must be homogenized.

Fish meal packed in 20 feet containers with *liners*: 20 increments of approximately 100 g each, per container, must be collected. The increments must be collected with an instrument with at least a 5 m reach, as per the following procedure:

- The container will be divided with 3 imaginary vertical lines, thus resulting in 4 segments of approximately 1.5 m. A total of 5 increments must be collected from the upper, mid and lower areas of each one of these sectors.
- At least 2,000 g of representative samples must be collected from each lot.
- The increments must be poured in a moisture tight, sterile and properly identified container, until completing the entire representative sample. Once in the laboratory, the sample must be homogenized.

Fish meal in bulk: The sampling process to be applied is called proportionate stratification sampling. It consists of drawing imaginary lines in the inspection batch to obtain inspection lots.

The inspection batch must be identified with the corresponding production codes in a visible area, to clearly identify the lots.

The determination of the number of increments to be collected considers the following:

- The verification of the number of tons declared by the company through the estimation of the number of tons that are part of each inspection batch (length, height, and width), considering that the density of the fish meal is equivalent to 0.4 - 0.6 ton/m^3.
- The subdivision of each inspection batch into 50-ton inspection lots, out of which a minimum of 50 increments will be collected.
- Once the lots are identified, the samples will be collected with a sampling spear, and the number of increments must be indicated as follows:
- The sampling points must be chosen at random and in a systematic way, in the upper part of the lot.
- The increments must be taken by inserting a spearing tube inverted and in a 45º to 50º and rotating it before withdrawal. This procedure must be repeated at approximately 1.3 - 1.5 m intervals apart, covering the entire surface of the lot.
- If the height of the lot exceeds the length of the spearing tube, or if it does not reach the bottom of the pile, the samples must be collected from the sides of each lot, at a height that the spear cannot reach. These increments must be collected with a separation and tilt similar to those mentioned before (1.3 – 1.5 m and 45º to 50º).
- If the height of the lot is equal to or higher than 3 m, the increments on the sides must be collected in an imaginary zig-zag line, and its upper edge must start at the height at which the spear could not be introduced through the upper part of the pile, and its lower edge must end in the areas where the spear was introduced on the sides, with the specified tilt, and where there is no risk of reaching the bottom of the lot.
- The quantity of the representative sample collected from each lot must be at least of 50 increments (no less than 8 kg).

The representative sample of each lot must be collected in a sterile container of a proper size and material, and it must allow homogenizing all the increments that comprise the sample. Once homogenized, the sample must be reduced to a minimum of 1,500 g and poured into a properly identified sterile or first use bag. It must be homogenized again in the laboratory.

If there is a lot with unfavorable results, the entire inspection batch will be considered to be positive, since the only purpose of this separation in lots is to help collect the samples.

On-line Sampling: The sampling method to be used corresponds to a random systematic procedure that takes place at the moment of packaging before sealing the sacks or containers.

The fish meal that is packed directly from the production line must be identified with the production date.

If it comes from fish meal stored in sacks or in bulk, it must keep its original identification. Fish meal of origin must be identified as follows:

- Inspection lot: Quantity of fish meal in any presentation of up to 50 tons, whose biological, physical and chemical characteristics are presumed to be uniform.
- In the case of meal packed in containers, the inspection lot corresponds to the container.
- Inspection batch: Quantity of fish meal in bulk comprised of one or more inspection lots of the same production date and which is set up forming a pile.
- Representative sample: Quantity of fish meal collected from an inspection lot which reflects its biological, chemical and physical characteristics.
- Increment: Quantity of fish meal collected from an inspection lot at one time with a probe or spear.
- Identification of the lot: The inspection lot must be identified with the production code, comprised of the date of production (numerical or alphanumeric system) followed by letters or digits that allow individualizing the inspection batches from the same date of production. This code must be printed in the sack or on the individual label. In the case of containers, the production code must be printed in a visible area.
- Identification of the inspection batch: It must include the production code, comprised of the date of production (numerical or alphanumeric system) followed by letters or digits that allow individualizing the inspection batches from the same date of production. Similarly, the tonnage of the batch must be specified and its inspection lots.

This sampling procedure is only effective for collecting samples for microbiological analyses.

- On-line sampling during the packing of sacks:

2 increments per ton must be collected, with a spoon or scoop with a minimum capacity of 25 g. The increments must be collected from different bags before they are sealed. The sacks to be sampled must be selected at random, determining its frequency based on the total estimated volume, keeping a continuous interval between the selected sacks.

Alternatively, the sample can be collected directly from the sack packing line, manually or through automatic dispensers.

At least 1,500 g of representative samples must be collected from each lot.

Handling the representative sample: The increments must be poured in a moisture tight, sterile and properly identified container, until completing the entire representative sample. Once in the laboratory, the sample must be homogenized.

- On-line sampling during the packing of 20 feet containers with *liners*:

Increments will be taken at random from the continuous flow of the filling machine. The time interval to obtain such increments must be determined based on the total estimated filling time of the container and considering the collection of one increment per ton.

At least 2,000 g of representative samples must be collected from each lot.
The increments must be poured in a moisture tight, sterile and properly identified container, until completing the entire representative sample. Once in the laboratory, the sample must be homogenized.

2.1.3.4 FISH OIL SAMPLING

The sampling procedures for each one of the fish oil presentations are described as follows:

a) Oil Stored in Tanks

For those oils stored in tanks, "n" samples must be extracted, as described in Section III, Chapter IV, Item 1, considering at least 2 collection strata (surface and bottom), thus using the proper tools. The volume to be extracted must correspond to that needed to conduct the required analyses.

Handling the representative sample: The partial samples, collected according to the "n" required sample, must be sent separately to the laboratory, in individual, moisture tight, sterile and properly identified containers. Once in the laboratory, these samples must be mixed to create a single sample.

b) Oil Stored in Gallons

For oils stored in drums, gallons or in other standard units, the samples of "n" gallons must be selected at random. The volume necessary to conduct the analyses required must be extracted from each one.

The partial samples, collected according to the "n" required sample, must be sent separately to the laboratory, in individual, moisture tight, sterile and properly identified containers. Once in the laboratory, these samples must be mixed to create a single sample.

2.1.3.5 SAMPLING FOR THE CONTROL OF PHARMACEUTICAL PRODUCTS, UNAUTHORIZED SUBSTANCES, PROHIBITED SUBSTANCES, AND CONTAMINANTS

The sampling procedures described below, consider all of the establishments where it is possible to carry out the control of pharmaceutical products, unauthorized and prohibited substances, that is to say, farms, processing plants, cold stores, among others.

When the sampling is carried out by SERNAPESCA, the establishment must provide the material for the sampling.

The samples must be sent under proper isolation and temperature conditions. At the moment of sampling, must be considered transparent bags without lithography and white gloves, avoiding colored materials. The label of the samples must be between two packages, never in direct contact with the sample. Only graphite pencil should be used.

When the sampling is carried out by SERNAPESCA, the establishment must provide the material for the sampling and dispatch of the sample throughout the productive cycle, as well as, they will be responsible for the shipment and for communicating to the laboratory the dispatch of the samples.

2.1.3.5.1 SAMPLING FOR THE CONTROL OF PHARMACEUTICAL PRODUCTS IN FARMS

The sampling for the control of pharmaceutical products residues in farms must be carried out by samplers of Export Fishery Products (EFP) authorized as required in the Chapter I of this Section.

The farm must define, before the sampling, the number of cages to harvest and their identification. Once the sampling has taken place, the person in charge will issue the Sampling Request for Harvest with one original and two copies, in accordance with the format established in Chapter II, Part III Annexes. The original version of the request will be dispatched by the sampler, together with the samples of the analysis entities, and the copies will be distributed to the sampling entity and the farm.

The Sampling Request for Harvest must be signed by the sampler, in the corresponding box of Item II in the document. The person responsible for the sanitary aspects of the farm must also have available at the moment of the sampling, a layout with the distribution of the cages in the farm and the estimated number of fish per cage. The sampler will mark in this layout the cages that were in fact sampled.

The pre-harvest sampling requests must be presented by the fish farming company interested in conducting such samples. These requests must be completed with clear information of the groups or cages identified for the sampling. Such request must be properly signed by the person appointed by the company for such purpose, and therefore no further changes in the reports from the laboratory will be accepted.

The Analysis entity must thoroughly review the documentation received together with the samples, verifying that it contains all the necessary information and that it is duly signed. Such entity must not accept incomplete or unsigned pre-harvest requests.

The following must be considered for the sampling:

a) Sampling Procedure:

- The authorized sampler must go to the farm, where the pre-harvest sampling request will be available.
- Once the cages to be sampled have been identified, a sampler will collect the samples that were previously described in the pre-harvest sampling request, as outlined in Section I, Chapter II.
- The sampler will be responsible for verifying that the request is complete and that the information provided by the farm is truthful. Afterward, it must fill in the necessary information in the "Samples Delivery and Sampling Report Form" and will mark the cages that were sampled in the layout available on the farm. Close attention must be paid when consigning the cages that were sampled, the number of samples collected from each cage and if it is a group sampling, clearly indicate the cages to be released.

- The sampling report must consign the weight in kg. of every fish obtained for the sampling. Fish can be weighed in the farm or in the analysis laboratory if they arrive in one piece. The purpose of this measurement is to control the fact that the fish used for the samples are the most representative from the cage. Therefore, the aim is for the fish to be sampled to be as closest to the estimated average weight for the cage.

b) Collecting the Sample:

Sample collection is a procedure that must completely ensure that it does not contaminate the environment, does not create cross-contamination between the samples and that it keeps a perfect traceability between the sample collected and the cage where it came from.

- The samples collected from the cages will be sent to the analysis laboratory in one piece. The live fish will be properly desensitized. The fish may not be bled, eviscerated or sliced in the farm unless the Sampling entity has the proper equipment to conduct this task and dispose of the remains properly. This procedure may only take place in the farm under conditions that guarantee that there will be no harm to the environment.
- If the farm does not have the proper conditions, the samples may be cut in a sampling entity authorized by SERNAPESCA to be then sent to the residues analysis laboratory. This process must be conducted following the procedures set forth by the Sampling Entity and according to the criteria herein.

c) Reduction of Samples:

- There must not be any type of cross-contamination between samples. For this, the Sampling Entity must implement a procedure where the tools used between samples and sampled groups are washed and switched.
- Samples must be handled without using gloves.
- Before cutting the fish, it must be completely eviscerated, as per the regular evisceration process.
- The reduction procedure must ensure that a standard sample is collected. For this, the fish will be cut vertically from its axis, behind the dorsal fin and in front of the anal fin. The piece to be sent to the analysis laboratory must necessarily include the fish's tail. (See figure 1).
- The samples for residues analysis must be comprised of a minimum of 400 gr. of muscle and skin.
- If the sample weighs less than 400 gr, the vertical cut must be made closer to the head of the fish so as to obtain the minimum of 400 gr. required. The limit is the entire fish, eviscerated and headless.
- If the eviscerated and headless fish weighs more than 400 gr. the necessary pieces must be taken until reaching the 400 gr. This will be considered as a single sample, and therefore, it must be packed and labeled as such.

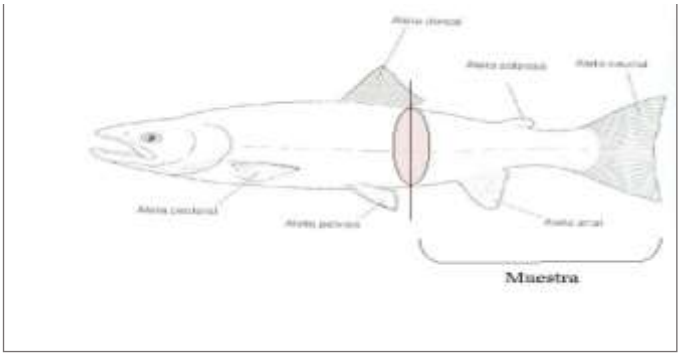

Figure 1: Salmon sample for residue analysis before harvest.

d) Packing and Delivering Samples:

- The samples will be double-packed and labeled individually, ensuring their traceability and quality when conducting the analyses.
- The packaging of the samples (bag, box or similar) must be of first use, clear and without any printing.
- The label of each sample must be placed between the two packages, and never in direct contact with the sample. Only graphite pencils may be used.
- The individual package must be sealed and must comply with the same specifications described in letter b).
- The samples must be sent under proper isolation and temperature conditions, thus guaranteeing their proper condition until they are received by the Analysis Entity.
- The final packaging must be sealed with the Sampling Entity's tape or seal.
- The label of the sample must indicate the number of the sample, species, number of the cage or origin, lot identification, farm, farming company, sampling date and the name of the authorized sampler.

e) Collecting Counter Samples:

- The pharmacological residues Analysis Entities are responsible for keeping a counter sample of each sample received under a Sample Request for Harvest from SERNAPESCA.
- The piece of muscle to be separated for each counter sample will come from each sample received. It must be collected in a clean way and with the lowest level of handling possible. The piece taken must be whole and must not be part of the processed sample. The osseous and cartilaginous tissue must be extracted, keeping only the muscle and skin.
- The storage of counter samples must be as described in Item 2.17 of this Chapter.
- The pre-harvest counter samples will be stored for a minimum of 60 business days.

f) Pre-harvest Sampling Procedure per Cage

The number of samples to be collected will depend on the treatment applied to the fish in the cage:

- In the case of treatments with oral pharmaceutical products (feed), a total of 7 samples (n=7) will be collected per cage. The samples must correspond to fish that are in the group (20%) of higher weight in the cage.
- If the treatment applied corresponds to injectable pharmaceutical products, a total of 10 samples (n=10) will be collected per cage. The samples must correspond to fish that are in the group (20%) of lower weight in the cage.

- In the case of treatments with injectable pharmaceutical products, combined with oral treatment with the same active principle, a total of 20 (n=20) samples will be collected. The samples must correspond to the groups of higher and lower weight inside the cage, in equal proportions (n=10 fish of lower weight and n=10 fish of highest weight).
- In the case injectable and oral treatments with different active principles, 17 samples (n=17) must be considered for each cage, out of which 7 samples (n=7) must correspond to fish that are in the highest weight group (20%) inside the cage and 10 samples (n=10) for fish that are in the lowest weight (20%) group inside the cage.
- In immersion anti-parasitic treatments (Cypermethrin), a total of 5 samples (n=5) will be collected per cage, regardless of the weight of the fish.

g) Pre-harvest Sampling Procedure per Group

To the extent that it is conducted per treatment group, a total of 59 samples (n=59) must be collected for every group of treated fish.

The collection of 59 samples must be equally distributed among all the cages of the group, comprising up to 12 cages. For groups comprised of more than 12 cages, these must be selected randomly, through a random sample selection process.

The considerations stated herein, are additional to the instructions described in Chapter III, Item 4 of this Section, Section IV, Chapter II, Item 1, and Section I, Chapter II of this Manual.

h) Pre-harvest Re-Sampling Procedure

If the re-sampling process corresponds to a sampling process previously carried out per cage, a total of 5 samples (n=5) will be collected, and the analyses required will correspond only to those pharmaceutical products detected in the first sampling.

When the re-sampling corresponds to a sampling conducted per group, a total of 29 samples (n=29) will be collected, which must be equally distributed among all the cages of the group, comprising up to 6 cages. For groups comprised of more than 6 cages, these must be selected randomly, through a random sample selection process. An analysis must only be carried out for the pharmaceutical products detected in the first sampling.

2.1.3.5.2 SAMPLING FOR THE CONTROL OF PROHIBITED AND UNAUTHORIZED SUBSTANCES IN FARMS

The sampling of prohibited and unauthorized substances in farms must be carried out by SERNAPESCA inspectors.

The sampling must be conducted taking 7 individual fish (n=7), which must be alive, healthy and being fed normally.

Each sample must weigh at least 400 grams. In the case of small fish, a sample may be comprised of several individuals.

For the delivery of the samples, the Sample Delivery and Sampling Report Form must be used, whose format is presented in Chapter II, Part III, Annexes. Such form must describe at least one cage, a lot, farm, and its code, company name, species, sampling date, the name of the SERNAPESCA inspector present during the sampling, name that identifies the sample and special remarks. A copy must be provided to the SERNAPESCA Office under the jurisdiction of the farm.

The SERNAPESCA inspector must seal the boxes with official numbered seals, which must be dispatched to the SERNAPESCA Verification Laboratories for the corresponding analysis. The farm must have, during the entire production cycle, all the necessary tools and equipment for collecting and delivering the sample.

The farms must include in the Declaration of Guarantee, the report number for Prohibited and Unauthorized Substances and its date of issuance, corresponding to the sampling in the fattening stage, or the annual sampling in the case of fish cultures, conducted by SERNAPESCA.

2.1.3.5.3 SAMPLING FOR THE CONTROL OF PHARMACEUTICAL PRODUCTS RESIDUES, UNAUTHORIZED SUBSTANCES, PROHIBITED SUBSTANCES, AND CONTAMINANTS IN PROCESSING PLANTS

The sampling of pharmaceutical products residues, prohibited and unauthorized substances in processing plants must be carried out by SERNAPESCA inspectors.

The verification samples may be collected at the entry of the raw material at the plant, during the process or on the finished product. The sampling must be conducted without prior notice and taking all the necessary precautions to ensure the traceability of the samples.

The samples, of at least 40 grams, must be of muscle with skin in natural proportions of fresh or frozen product that has not been subjected to chemical or heat treatments that may interfere with the analyses and chromatographic readings of the results.

The 10 samples must come from the same production lot (each sample must be collected from different fish).

The inspector will conduct a focused sampling, being able to be assisted by the person in charge of the Quality Assurance Program (QAP), or by a sampler authorized by SERNAPESCA, so as to select the lots at greater risk. For this, it may consider some information, such as, the use of currently unknown substances, diseases that suddenly arose in specific areas, an indication of fraudulent activities, information on previous positive results, etc.

The boxes must also be sealed with an official numbered seal, for its further delivery to the SERNAPESCA Verification Laboratory.

The samples must be sent to the Verification Laboratory of SERNAPESCA, with the Sample Delivery and Sampling Report Form, whose format is shown in Chapter II, Part III, Annexes, identifying the cage, lot, farm and its code, company, species, sampling date, processing establishment of the product and authorization number, name of the SERNAPESCA inspector present during the sampling, a number that identifies the sample and any special remarks, as appropriate.

When the sample corresponds to a corrective action, this information must be entered in the form sent to the SERNAPESCA Verification Laboratory.

2.1.3.6 SEAWEEDS SAMPLING

The sampling for seaweeds exports must be conducted according to the protocols set forth in Chilean Standard NCh43.Of1961 "Selection of Samples at Random."

2.1.3.7 WATERS SAMPLING

This procedure will apply to all the annual, and standard water samplings carried out by fishery products export establishments.

The frequency of the samplings and corresponding water and ice samplings are described in Section II of this Manual.

The collection, preservation and storage procedure for water samples must be conducted mainly considering the recommendations of Chilean Standard NCh 409/2 Of.2004 and the Potable Water Analysis Methods Manual (Métodos de Análisis del Agua Potable), 2nd edition, July 2007.

The following must be considered for physical, chemical and microbiological analyses:

a) All samples must be properly labeled and sent to the laboratory as soon as possible and refrigerated, so as to keep a proper temperature, which must not exceed 10 C°, and avoiding freezing. For the specific case of microbiological samples, no more than 20 hours must elapse between the collection and the analysis. Once the samples are received, they must be maintained at a temperature between 1 and 4 C° until their analysis; this requirement is not applicable when the sample is analyzed immediately.

b) The containers to be used to take the samples for physical and chemical analyses may be of neutral glass or polyethylene. The procedure for washing these containers and their caps and overseals must be done in general, with a special detergent designed for laboratory glassware, followed by at least two rinses with abundant tap water, and then at least three more rinses with Type III reagents water for analysis or higher. Then, they must be washed with acids or solvents so as to eliminate any substances that may interfere with the tests.

c) The containers used in microbiological assays must also be sterilized before their use, before protecting their neck and cap with aluminum foil and a layer of brown *kraft* paper. Sterilization can be done in two ways depending on the type and resistance of the material, as follows:

- With dry heat in a sterilization oven for 1 hour at 170 C° ± 10 C°, after adding the corresponding preservative.
- With moist heat in an autoclave: for 15 minutes at 121 C° ± 2 C°, after adding the corresponding preservative.

0.1 ml of sodium thiosulfate solution at 10% m/v for every 120 ml of the sample must be added to the container before the sterilization.

Any microbiological containers that are reused must be decontaminated before being reused with steam in an autoclave at a temperature of 121 C° ± 2 C°, for at least 30 minutes, to be able to discard original samples.

The specific procedures for taking samples are described as follows:

a) Sampling for physical analyses: The minimum volume of the sample required to conduct the physical analysis is 500 ml. In the case of those containers that do not have preservatives, the water to be sampled must run for at least one minute, and the container must be rinsed at least three times before taking the sample.

b) Sampling for chemical analyses: The minimum volume of the sample required to conduct the chemical analysis is 5 liters. Before collecting the sample, water must run for at least one

minute, and the determination of the temperature and pH must be done when collecting the sample.

c) Sampling for microbiological analyses: The volume of the samples to conduct microbiological analyses must be of 200 ml.

Water must run for at least one minute before collecting the sample.

When collecting the sample, special care must be taken so as not to contaminate the opening of the container; it must be filled without rinsing up to 3/4 of its capacity and closed immediately. Once the sample has been collected, it must be clearly identified and delivered without undue delay to the laboratory.

2.1.3.8 CONTACT SURFACE SAMPLING

This procedure will apply to all the surface samplings to determine the presence of *Listeria monocytogenes*.

a) Tools:

- Sterilized in cellulose or polyurethane individual sponges.
- Sterile plastic bag to hold the sponge after the sampling.
- Sterile disposable gloves.
- Dilution solution: Buffer solution, nutrient broth or sterilized peptone water (0.1%).
- Neutralizers according to the disinfectant used on the surface. The neutralizer must be added to the dilution solution (ISO 18.593).

b) Sampling Procedure:

- The sampler must wash and sanitize his hands before putting on gloves.
- Open the package containing the sterile sponge.
- Aseptically place the solution and the neutralizer inside the sterile bag with the sponge.
- Wet the sponge in the solution 10 (ml) and remove any excess.
- Carefully take the sponge out of the bag.
- Conduct the sampling process in a defined area (of at least 100 cm^2, preferably around 1,000 cm^2).
- Rub the sponge horizontally, vertically and diagonally 10 times respectively turning the sponge every time.
- Once the sampling has been completed, store the sponge in a sterile bag, removing the air from the inside.

2.1.3.9 SAMPLING METHODS FOR COLD STORE AIR AND WALLS

This procedure will be applicable for all air and cold room samplings in the context of the Sanitation Operational Programs (SOP), as stated in Section II of the Food Safety and Certification Manual.

The following stated in Annex 7 must be complied to conduct the process: Instructions for the definition and assessment of mold in the air and in the walls of cold stores from the Sanitary Standards for Cold Stores (approved by the State General Sanitary Doctor of the USSR on 09.29.1988, N4695-88) of the Eurasian Economic Union.

For the purpose of this sample, instead of using a metal brush scraper, for collecting wall samples, as stated by the previously mentioned standard, a sponge or cotton balls are to be used, considering that the surfaces of the cold stores are of pre-lacquered steel and not of stainless steel.

One sponge or cotton ball must be used per wall. If using a sponge, this must of a size, shape and absorption capacity, in which the four sponges together allow to conduct the initial dilution of the sample according to what is stated in the sanitary standard for cold stores.

2.1.3.10 INSPECTION AND SAMPLING FOR EXPORT FISHERY AND AQUACULTURE PRODUCTS SHIPMENTS WITH DESTINATION TO THE EURASIAN ECONOMIC UNION (EEU) AND BRAZIL

The inspection consists of the thorough verification and identification of the products to be shipped, to ensure that they match the description of the products included in the *packing list* or AOCS. In addition to verifying the compliance with the pre-shipping requirements such as the temperature of the product, the condition of the packaging, as well as the conditions of stacking, cleanliness of the container, as appropriate, among other information described in Section IV, Chapter II, Item 1 and Section III, Chapter III.

The following must be complied to carry out the inspection and sampling procedure:

- In the cold store, the sampler must select from the products that comprise the *packing list* or AOCS n=13 boxes for temperature control. In the case of salmonids, n includes the control of injuries.
- The temperature measurement and the control of injuries will be conducted in the sampling room.
- Once the boxes have been sampled, they must be closed immediately with the adhesive tape of the sampling entity and returned to the cold store to avoid any increases in the temperature of the product.
- If there is not a condition in the regulation that does not comply with the standard, the next step will be the verification and detailed identification of the products to be shipped.
- All safeguards must be taken in all the stages, so as to avoid any unnecessary increases in the temperature of the products.

2.1.4 LABELING OF SAMPLING CONTAINERS

The containers must be identified with labels. The samples must be labeled with a code or consecutive numbers to identify them unequivocally until the corresponding analyses take place.

The container with all the labeled samples must include at least the following information:

- Type of product.
- Key or production date.
- Number of samples with their corresponding identification.
- Time and date of the sampling.
- SERNAPESCA Sampling and Analysis Request Number, BMSP Samples Delivery Form or Samples Delivery Form for QAP Verification, as appropriate.

In the particular case of samples taken for the analysis of marine biotoxins, either in the context of the End Product Control, the Quality Assurance Program or the Bivalve Mollusks Sanitation Program, the box that will carry the samples, must indicate on the outside in a clear and large letter, the following sentence: "SAMPLES FOR MARINE BIOTOXINS ANALYSIS". The above, with the aim of identify the box easily when entering the laboratory, without need to open it.

2.1.5 TRANSPORTATION AND STORAGE OF SAMPLES

Since the handling of samples can lead to changes in their microbiological, physical-chemical and organoleptic condition in a larger or lower extent, they must be transported to the laboratory as soon as possible and in proper preservation or storage conditions, if necessary.

a) Refrigerated Fresh Products

The samples must comply with the following requirements:

- They must be kept at a temperature of 0 to 5 C°.
- They must be transported in an insulated container with ice or other refrigerants (*gel pack*).
- The containers must be kept in a vertical position, and the product must not make contact with the refrigerant.
- The timeframe between the sampling and the arrival of the samples to the laboratory must not exceed 36 hours for these type of products.
- For fresh fish samples in farms, the Transportation and Storage of the sample described in Annex I must be considered.

b) Frozen Products

The samples must comply with the following requirements:

- They must be received in the analysis entity, at a maximum temperature of -5 C°.
- They must be promptly transported to the laboratory in isothermal boxes suitable for the size and quantity of the stored sample, with refrigerant, in no more than 36 hours.

c) Refrigerated and Frozen Fresh Products

The laboratory may analyze frozen and refrigerated samples with a temperature above -5 C° y 5 C°, respectively, to the extent that:

- The timeframe between the sampling and the arrival of the samples at the laboratory does not exceed 36 hours.
- The timeframe between the sampling and the arrival of the samples at the laboratory does not exceed 48 hours for samples coming from Chiloé and the regions of Aysén and Magallanes.
- The samples are in optimal organoleptic conditions.

Whether or not the sample is accepted or rejected based on these conditions, the analysis entity must confirm this situation with the SERNAPESCA office of the region to which the processing establishment belongs, in writing. If deemed necessary, both the inspector of SERNAPESCA, as well as the laboratory may ratify the acceptance or rejection of the samples with the plant.

d) Meal and Oil from Fishery Products

The samples must be in optimal organoleptic conditions as stated in Section III, Chapter IV, Item 1.

e) General Considerations:

- The samples may be stored, before sending them to the analysis entity, in the chambers of the processing plants or cold stores approved by SERNAPESCA for no more than 3 consecutive calendar days. The delivery date must be clearly indicated in the QAP Verification Samples Delivery Form. This procedure must be previously agreed with the Inspector of SERNAPESCA responsible for the plant.
- Once the frozen samples have been received, the analysis entity will have a maximum of 5 consecutive calendar days to start conducting the corresponding analyses.
- For those laboratories that conduct marine toxins analyses, the received samples under the context of the Quality Assurance Program, with a FEM QAP form, must have the same priority of

the samples received under the context of Bivalve Mollusks Sanitation Program, that is, a top priority.

- The laboratory may not accept samples on Fridays or on holiday eves when this has not been previously agreed.
- The sample sent to the SERNAPESCA Verification Laboratory must be sealed with adhesive tape with the SERNAPESCA logo.
- All samples must be sent to the analysis laboratory in sealed packages and boxes. This is to guarantee that they have not been adulterated between the sampling process and their reception at the laboratory.
- The sampling entity will be responsible for keeping the samples in optimum conditions, from the collection of the samples until their arrival at the analysis laboratory.
- When samples need to be analyzed in different laboratories due to the outsourcing of analyses (subcontracting or sending out), these must be sent initially to the laboratory conducting the microbiological analyses. If the outsourcing were between two laboratories that conduct microbiological analyses, the samples must be sent simultaneously to each one of the laboratories together with their corresponding SERNAPESCA official forms, so as to avoid their deterioration.

2.1.6 SAMPLING REPORT

A complete sampling report must be written and signed by the person responsible for it, including at least, the following information:

a. Sampling report number.
b. Audit Number and Document Number, in the case of digital analyses reports with an electronic signature with a Certified Electronic Document (CED).
c. Number of pages.
d. Date of issuance.
e. Name and entity of the authorized sampler.
f. Date, start time, end time, and location of the sampling.
g. Description of the sampled product (as per SIEP tables*).
h. Name and category of the production company.
i. Name of the company's representative present during the sampling.
j. Declared destination of the product.
k. Number and size of the units that comprise the lot.
l. Key or production date of the lot.
m. Expiration date or shelf-life of the product (as appropriate).
n. Plan or layout of the location and distribution of the sampled lots, for bulk meal.
o. Sampling regulation used.
p. SERNAPESCA Sampling Request Number, accordingly.
q. QAP Verification Samples Delivery Form, as appropriate.
r. BMSP Sampling and Analysis Form Number, as appropriate.
s. Size, quantity and reference code of the samples, collected per each key.
t. The temperature of the product at sampling, as appropriate.
u. The temperature of the place of storage, as appropriate.
v. Name and address of the laboratory that will conduct the analyses.
w. Types of analyses to be conducted.
x. Indicate the presence or absence of identification for primary and secondary packaging with the code of the establishment and the country of origin (Chile).

In the specific case of the monitoring of natural banks of the BMSP, at least the following information must be considered:

y. Name and signature of the sampler.
z. Name and registration number of the vessel.
aa. Name of the divers that are part of the sampling process.
bb. Date of issuance of the sailing permit and the Port Authority issuing it.

The report must also include a notes section for informing about situations, conditions or circumstances that may have had an influence in the sampling or that are to be considered for a future analysis of samples, for instance, the condition of the packages, breakages, the presence of moist, etc.

2.1.7 INSPECTION REPORT FOR REENTERING FISHERY PRODUCTS

The inspection for reentering fishery products report must be written by the responsible authorized sampler in charge of conducting the process, including the information described in Item 2.1.6 of this chapter, in addition to the following:

a. Background information:
   i. Description of the product (type and presentation).
   ii. Exporter.
   iii. Container Number.
   iv. Seal Number.
   v. The hygienic conditions of the container.
   vi. The general condition of the product.
   vii. Stacking conditions.
   viii. Packaging condition.
   ix. Date of inspection.
   x. Start and end time of the inspection.
   xi. Location of the inspection.
   xii. The temperature of the container when it was opened (as per Section IV, Chapter II, Item 2).
   xiii. Labeling and its condition (as per Section II, Chapter I, Item 2.2).
   xiv. SIPP Number.
   xv. NEPPEX Number.
   xvi. Name of the sampler.
   xvii. Sampling entity.
b. *Packing list*:
   i. Type of product.
   ii. Presentation of the product.
   iii. Producer (name and number).
   iv. Production date (and/or code and/or traceability, if applicable).
   v. Expiration date.
   vi. Condition of the packaging (primary and secondary).
   vii. Number of boxes.
   viii. Net weight.
c. Additional information:
   i. SIPP copy.
   ii. NEPPEX copy.
   iii. Thermal charts or visual control of side seams, as appropriate and upon request (as per Section II, Chapter V, Item 2).
   iv. A photographic record of the inspection.
   v. Results of the analyses, if applicable.

d. Results of the inspection:
   i. Table that summarizes the *packing list* information compared with the information included in the NEPPEX, in the case of inconsistencies.
   ii. Conclusions on the general condition of the product, based on the information collected.
   iii. Analyses results evaluation, based on the standards of the SERNAPESCA manuals (Section III, Chapter IV, Item 1 y Section II, Chapter II, Item 2).

2.1.8 INSPECTION AND SAMPLING PROCEDURE FOR EXPORT FISHERY AND AQUACULTURE PRODUCTS SHIPMENTS WITH DESTINATION TO THE EURASIAN ECONOMIC UNION (EEU) AND BRAZIL

The inspection and sampling report for export fishery and aquaculture products shipments with destination to the Eurasian Economic Union (EEU) must be written by the responsible authorized sampler in charge of conducting the process, including the information described in Item 2.1.6 of this chapter, in addition to the following:

a. Background information:
   i. Exporter.
   ii. Container Number.
   iii. Seal Number.
   iv. Hygienic conditions of the container.
   v. General condition of the product.
   vi. Stacking conditions.
   vii. Packaging condition.
   viii. Date of inspection.
   ix. Start and end time of the inspection.
   x. Location of the inspection.
   xi. Labeling and its condition (for the E.E.U. the date format is (day, month, year) according to Sanpin 2.3.4.050-96 standard).
   xii. Name of the sampler.
   xiii. Sampling entity.
   xiv. NEPPEX Number (processed by SISCOMEX).
   xv. AOCS Number (as appropriate).
b. *Packing list:*
   i. Type of product.
   ii. Presentation of the product.
   iii. Producer (name and number).
   iv. Production date and code (and/or traceability, if applicable).
   v. Expiration date.
   vi. Condition of the packaging (primary and secondary).
   vii. Number of boxes.
   viii. Net weight.
c. Temperature control:
   i. Indicate the results of the sampled boxes and their details.
d. Injuries control:
   i. Indicate the results of the sampled boxes and their details.
e. Additional information:
   i. A photographic record of the inspection only for "unfit" results.
f. Results of the inspection:
   i. A table summarizing the information of the shipped product compared to the information included in the AOCS or *Packing list,* as appropriate.

ii. Conclusion on the general condition of the product, based on the information collected, indicating if the product is fit or unfit to be exported to the EEU and Brazil.

2.1.9 COLLECTION AND STORAGE OF THE COUNTER SAMPLE

The laboratory will be responsible for obtaining the counter sample intended for chemical analysis from samples collected in the Certification Program (SMAE), Quality Assurance (FEM, QAP) and the Residues Control Program, and will be responsible for obtaining the counter sample intended for the instrumental analysis of lipophilic toxins, from the homogenization of samples coming from the Bivalve Mollusks Sanitation Program (BMSP).

The collection of counter samples will be required for the following analyte-product pairs:

1) Metals in hydrobiological products (except for fish meal and oil, and products extracted in the context of the Bivalve Mollusks Sanitation Program (BMSP).
2) Pharmaceutical products residues in farmed fish.
3) Contaminants in farmed fish.
4) Unauthorized substances in farmed fish.
5) Prohibited substances in farmed fish.
6) Lipophilic toxins for the instrumental analysis by LC-MS/MS, in bivalve mollusks, echinoderms, and tunicates.

The laboratory must also consider the following:

- The counter sample will be obtained from the sample, and its quantity must be enough so as to conduct the analysis again.
- The fresh and cooled refrigerated products coming from the control conducted in the Certification and Quality Assurance Program, must be considered when obtaining the counter samples.
- The collection of counter samples for canned products will take place in the laboratory and from the sample. Therefore it will not be necessary to extract additional counter samples apart from those extracted when conducting the sampling.
- The preservation period for the counter samples, regardless of their presentation, will be at least 60 business days from the issuance of the laboratory analysis report. The storage temperature of the counter samples must be equal to or lower than -18 C°.
- The counter samples may not be used to revalidate the analyses. The revalidation of analyses does not require counter samples.
- The counter samples must be stored in sealed containers that do not allow their adulteration.
- The counter sample must be labeled in the same way as the samples, so as to be able to identify them at any time. There must be a record in a book with numbered pages that identifies each counter sample and its exact location.
- The counter sample will be used exclusively by SERNAPESCA, and the analysis entity will be responsible for maintaining it in proper conditions during the corresponding period.
- The counter samples may be discarded once the maintenance period has concluded. For this, a Counter Sample Disposal Record must be written, including, among other information, the type of product, the keys, the SERNAPESCA Form Number (SMAE, FEM QAP, FEM BMSP) and the discard date.
- In the case of the lipophilic toxins instrumental analysis, the counter samples will be generated from the homogenization of samples coming from the Bivalve Mollusks Sanitation Program (BMSP).

3. SENSORY EVALUATION OF LIVE OR CHILLED-REFRIGERATED FISHERY PRODUCTS

The purpose of this standard is to establish the procedure to determine the sensory characteristics of a fishery product through the sensory examination of a sample of the product.

3.1 SCOPE

The standard establishes that the sampling and sensory evaluation procedure must be applied to chilled, refrigerated or live export fishery products.

3.2 SAMPLING PROCEDURES

Before conducting the sampling and evaluation, the authorized sampler must have the original version of the Notification of Shipment of Export Fishery Products, as set forth in Item 1 of this Chapter.

3.2.1 BATCH IDENTIFICATION

The authorized sampler must verify that the batch to be evaluated matches the description included in the Notification of Shipment of Export Fishery Products. Other aspects to be verified include, among others:

a) Number of boxes: All the boxes available to be selected for evaluation.
b) Labeling: It must be verified that both primary and secondary packaging, as appropriate, contain the following information: production plant code, the word CHILE, production key, species (common and scientific name), presentation or type of production, *packing list* number.

3.2.2 COLLECTING THE SAMPLE

The sampling unit is comprised of each one of the boxes of the lot or lots that are part of the export batch. The boxes of the batch or part of it may not be placed in wire containers in shipping pallets and or in closed tin containers when selecting the boxes.

Samples must be extracted from each production date and product described in the Notification of Shipment of Export Fishery Products, as per the sampling plans described in Section III, Chapter IV, Item 1.

The samples can be collected at random from the entire lot to be sampled (as per Chilean Standard NCh43). This procedure may be conducted while unloading the product at its arrival at the port of loading or once the batch or lot has been unloaded and placed in pallets before loading.

The selected units or boxes must be completely evaluated according to their sampling plan.

3.2.3 IDENTIFICATION OF SAMPLING UNITS

The sampled units of each lot that comprises the batch must be immediately marked before or after being selected for examination, with labels or marking them with permanent ink. The label or mark must include the number of the sample's unit and the number of the corresponding Notification of Shipment of Export Fishery Products, so as to unequivocally identify the sample.

3.3 EVALUATION PROCEDURE

3.3.1 INSTRUMENTS AND SUPPLIES

To conduct the sensory evaluation, the Sampler must have the following instruments and supplies:

- A thermometer suitable for the type of product to be sampled. Its accuracy must be periodically checked against a duly calibrated standard thermometer.
- 70% alcohol to disinfect the thermometer.
- Sterile cotton.
- First-use disposable gloves.
- Adhesive tape with the name of the entity.
- Apron.
- Hat or cap.
- Container to discard waste material.
- Chilean Standard NCh43 "Selection of Samples at Random".

3.3.2 SENSORY EVALUATION PROCEDURE

The sensory evaluation will be conducted by an Official Sampler of Export Fishery Products, who must comply with the minimum sanitary requirements regarding his health condition, that is to say, that does not have any contagious bacterial or viral diseases, and that does not have any open wounds. Similarly, this person must undergo a very thorough process of personal hygiene while carrying out tasks using protective garments such as caps or hats that cover all the hair and an apron. Accessories or jewelry must not be used while handling products, nails must be kept short, clean and without nail polish (Article 56, Health Code).

To handle the products to be evaluated, new, disposable gloves must be used, which must only touch the product to be evaluated and must be changed when examining the boxes of other shipments. The use of gloves will not release the sampler of the obligation of washing his/her hands thoroughly before handling the product.

The evaluation of the sampling units must be conducted under proper hygienic conditions so that the product is not affected from the sanitary point of view.

The thermometer for controlling the temperature of the product must be disinfected before its use, with 70% alcohol and drying it with sterile cotton. Similarly, it must be disinfected before controlling the temperature of each box evaluated.

A cutter must be used for opening the seal of the boxes. The sensory evaluation must be conducted in the collected samples, controlling the parameters mentioned in Section III, Chapter IV, Item 1, as well as the sanitary condition of the containers and the refrigeration system of the means of transportation.

3.3.3 CLOSING THE BOXES

Once the evaluation has been conducted, each box or sampling unit must be closed and sealed with the entity's adhesive tape.

3.3.4 ISSUING THE SENSORY EVALUATION RECORD

Immediately after finishing the evaluation, the sampler will issue the Evaluation Record, one original version, and two copies. The original version must be delivered to the interested party, and the copies must be delivered to SERNAPESCA and the Sampling Entity, respectively.

The record must include, at least, the following information:

a. Sensory Evaluation Record Number.
b. Name and entity of the authorized sample.
c. Identification of the product, species and production line.
d. Producer (as appropriate).
e. Exporter.
f. Location of the evaluation.
g. Time and date of the Sensory Evaluation.
h. Total net kilograms.
i. Production keys, the number of boxes per key and number of boxes inspected per key.
j. Samples weight.
k. Identification of primary and secondary packaging, including the word CHILE, production plant code, production key, species (common and scientific name), type of production or presentation, and *packing list* number.
l. Waybill.
m. License plate number of the truck.
n. Physical and sensory characteristics of the product (according to Section III, Chapter IV, Item 1).
o. Results of the sensory evaluation, based on the physical and sensory characteristics of the product (according to Chapter II, Item 3).
p. Product classification.
q. Signature of the authorized sampler.

It must also include a remarks section that includes any aspects related to the evaluation procedure.

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CHAPTER III. ANALYSIS PROCEDURES AND METHODS

1. ADMINISTRATIVE PROCEDURES FOR ANALYSIS ENTITIES

1.1 SCOPE AND FIELD OF APPLICATION

This section includes the procedures to be followed by the officials of SERNAPESCA, as well as by the staff recognized by the Service, that are part of the authorized entities, for conducting the sampling, analysis and organoleptic evaluation of export fishery products.

It addresses the procedures to be followed for Certification, Quality Assurance, Bivalve Mollusks Sanitation and Residues Control Programs.

1.2 PROCEDURES RELATED TO THE CERTIFICATION PROGRAM (CER)

The analysis entity must check the information included in the Request for Sampling and Analysis for Export and in the Sampling Report so that it matches the samples received for analysis. Similarly, it must make sure that the samples are in proper conditions to start the corresponding analyses. If during the sampling process a situation that may have affected the condition of the samples arose, it must be taken into account before starting the analyses.

The transportation of samples to the laboratory must be conducted according to the instructions provided in Chapter II, Item 2. If the samples were not received in proper conditions for their analysis, the entity must inform the situation to the interested party and the regional office of SERNAPESCA to schedule a new sampling with the Sampling Entity, as soon as possible.

The organoleptic, chemical and microbiological analyses must be determined based on the corresponding destination market, as set forth in Section III, Chapter IV; and these must be carried out according to the methodologies described in Items 2, 3 and 6 of this Chapter. For chemical analyses, a counter sample must be obtained from the sample received beforehand, and it will be used only by SERNAPESCA to solve controversies.

The laboratories that need to outsource their analyses, either through outsourcing or sending them to other laboratories, must comply with the instructions described in Chapter I, Item 1.5.j. In addition, the laboratories that receive the counter samples will be responsible for storing the counter samples.

The terms established by the laboratory from the reception of the sample until starting the analyses, as well as their storage conditions, must agree with the regulations for analysis methodologies and the type of product, and may not affect in any case the final results of the analyses.

Once the analyses have been conducted, the entity must issue an Analysis Report providing the information set forth in Item 1.5. Those reports that must be sent directly to SERNAPESCA, as established by the corresponding program, must be sent within 48 hours after finishing the analysis.

If there are any unfavorable results in the analyses or if they fall outside the parameters set by SERNAPESCA regarding products, these must be immediately reported (within the next 12 hours) to the National Directorate of the Service and the interested party, as set forth in Item 1.6 of this

Chapter. The National Directorate will inform the region of origin of the producer and the certifying regions.

1.3 PROCEDURES RELATIVE TO THE QUALITY ASSURANCE PROGRAM (QAP)

1.3.1 ANALYSIS PROCEDURES FOR SERNAPESCA'S VERIFICATION

The SERNAPESCA Verification Laboratory must review the information included in the QAP Verification Samples Delivery Form (Part III, Annexes, Chapter II) so that it matches the samples received for analysis. Similarly, it must make sure that the samples are in proper conditions to start the corresponding analyses. If the samples have not been received in proper conditions for their analysis, the entity must inform this situation to SERNAPESCA, to carry out a new sampling if deemed necessary.

It must conduct the procedures described according to the form above, through the use of the official analysis methodologies of SERNAPESCA, as stated in Items 2, 3 and 6 of this Chapter. For chemical analyses, a counter sample must be obtained from the sample received beforehand, and it will be used only by SERNAPESCA to solve controversies.
The laboratories that need to outsource their analyses, either through outsourcing or sending them to other laboratories, must comply with the instructions described in Chapter I, Item 1.5.j.

In addition, the laboratories that receive the counter samples will be responsible for storing the counter samples.

Once the analyses have been conducted, the entity must issue an Analysis Report providing the information set forth in Item 1.5. Said report must be sent to the National Directorate of the Service, within 48 hours after obtaining the results, with a copy to the interested party, which must be filed by the processing establishment. If the report cannot be sent within the expected term, it must be immediately informed to the National Directorate of the Service and the interested party.

If there are any unfavorable results in the analyses or if they fall outside the parameters set by SERNAPESCA in terms of products, these must be immediately reported (within the next 12 hours) to the National Directorate of the Service and the interested party, as set forth in Item 1.6 of this Chapter, without waiting to complete all the analyses described in the QAP Verification Samples Delivery Form. The National Directorate will inform the region of origin of the producer and the certifying regions.

1.3.2 VERIFICATION ANALYSIS PROCEDURES AT THE SERVICES LABORATORY

The Services Laboratory must review the information included in the QAP Verification Samples Delivery Form (Part III, Annexes, Chapter II) so that it matches the samples received for analysis. Similarly, it must make sure that the samples are in proper conditions to start the corresponding analyses. If the samples have not been received in proper conditions for their analysis, the entity must inform this situation to the interested party, to carry out a new sampling if deemed necessary.

It must conduct the procedures described in the FEM, through the use of the official analysis methodologies of SERNAPESCA, as stated in Items 2 and 6 of this Chapter.

Once the analyses have been conducted, the entity must issue an Analysis Report providing the information outlined in Item 1.5 of this Chapter. Said report must be filed by the processing establishment.

If there are any unfavorable results in the analyses or if they fall outside the parameters set by SERNAPESCA in terms of products, these must be immediately reported (within the next 12 hours) to the National Directorate of the Service and the interested party, as set forth in Item 1.6 of this Chapter, without waiting to complete all the analyses described in the QAP Verification Samples Delivery Form. The National Directorate will inform the region of origin of the producer and the certifying regions.

1.4 PROCEDURES RELATIVE TO THE CONTROLOF PHARMACEUTICAL PRODUCTS RESIDUES, PROHIBITED SUBSTANCES, UNAUTHORIZED SUBSTANCES AND CONTAMINANTS

The analyses of residues and contaminants to support the Declaration of Guarantee must be conducted according to the instructions set forth in Section I, Chapter II and Item 4 of this Chapter.

The samples must be sent to the laboratories authorized by SERNAPESCA, included in the List of Analysis Entities, under proper conditions, as set forth in Chapter II, Item 2.

1.4.1 ANALYSIS PROCEDURES FOR SERNAPESCA'S VERIFICATION

For SERNAPESCA to conduct the Verification sampling, the samples must be sent, under proper insulation and temperature conditions, directly to the Verification Laboratory of the Program, as described in the List of Analysis Entities. The samples must be sent together with the QAP Verification Samples Delivery Form (FEM-QAP).

1.4.2 ANALYSIS PROCEDURES AT THE SERVICES LABORATORY

The authorized laboratory must verify the information accompanying the samples received for analysis. Similarly, it must make sure that the samples are in proper conditions to start the corresponding analyses. If the samples have not been received in proper conditions for their analysis, the entity must inform this situation to the interested party, with a copy to the SERNAPESCA National Directorate, to carry out a new sampling if deemed necessary.

It must conduct the procedures described according to the analysis methodologies authorized by SERNAPESCA, as per the requirements set forth in Item 4 of this Chapter.

Once the analyses have been conducted, the entity must issue an Analysis Report providing the information outlined in Item 1.5 of this Chapter.

If the results of the analyses are unfavorable or if they fall outside the parameters set forth by SERNAPESCA in terms of the product, for samples of the Residues Official Verifications Program, samples of the Prohibited Substances, Contaminants and Unauthorized Substances Control Program or for samples coming from the Certification Program, the National Directorate of the Service must be informed of the situation immediately and no later than 12 hours, as described in Item 1.6 of this Chapter. The National Directorate will inform the region of origin of the producer and the certifying regions.

If the results of the analyses are unfavorable or if they fall outside the parameters established by SERNAPESCA, for pre-harvest samples of the Residues Control Program, these must be informed to the National Directorate of the Service before 30 days and the laboratory's monthly activity statistics template may be attached, as set forth in Item 1.6 of this Chapter.

1.5 ANALYSIS REPORT

The analysis report must be written and signed by the person responsible for it, including at least, the following information:

a. Analysis report number.
b. Audit Number and Document Number, in the case of digital analyses reports with an electronic signature with a Certified Electronic Document (CED).
c. SERNAPESCA Sampling and Analysis Request Number, QAP Verification Samples Delivery Form Number, Sample Request for Harvest Number or BMSP Sampling and Analysis Form Number, as appropriate.
d. Name and location of the entity.
e. Number of pages.
f. Date of issuance.
g. Requestor of the analyses.
h. Producer. Name of the farm or processing plant and SERNAPESCA code, as appropriate.
i. Type of product, indicating the production line and scientific name of the species.
j. Size of the lot and key(s) of the product, or cage or pond number and treatment group, as appropriate.
k. Number of samples and identification.
l. Number, identification of the units that comprise the counter samples and the place of storage.
m. Sampling entity.
n. Sampling date.
o. Date of entrance of the samples in the laboratories.
p. Place of storage of the counter samples.
q. Analyses conducted.
r. Start date and time of each analysis.
s. End date and time of the all the analyses including the results report.
t. Results of the analyses.
u. The methodology used, their corresponding detection limits and quantification, as appropriate.
v. Authorized signatures.
w. It must also include a remarks/notes section that describes any aspects related to the analysis procedure.
x. Resampling (Yes/No).
y. Corrective Actions (Yes/No).

1. Requirements for issuing results reports:
    a. The means of issuing the results analysis report must guarantee, to the extent possible, that the information that it includes cannot be adulterated. Safeguards must be applied especially when sending reports in electronic formats.
    b. "Temporary," "preliminary" or "internal control" reports cannot be used for certification purposes and must be easily distinguished from final reports, using different codes.
    c. The code or number identifying the analysis report must be unique. If it is necessary to modify the information included in a results report, a new report may be issued, only under the express authorization of SERNAPESCA.

1.6 RESULTS ANALYSIS REPORT OF ANALYSES THAT HAVE NOT COMPLIED WITH SERNAPESCA'S STANDARDS (UNFAVORABLE RESULTS)

The authorized Analysis Entities must have a procedure in their Quality Manual to ensure the timely delivery of results reports of analyses that do not comply with the standards of SERNAPESCA regarding products. The purpose of these procedures is to guarantee that in the extent that unfavorable results take place, these will be informed immediately to SERNAPESCA and to the interested party, in a standard report format that allows adopting the corresponding measures in an accurate and timely manner. In the case of the Bivalve Mollusks Sanitation Program, these must be sent within 2 hours after getting the results. For the rest of the programs of the Service, they must be sent within 12 hours. The laboratory carrying out the assay is responsible for informing about an unfavorable result of an analysis.

The procedure to be included in the Quality Manual of the Analysis Entity must comply at least with the following:

1) The purpose of the procedure must agree with the purpose presented in this document.
2) The scope of the procedure comprises all the analyses related to the SERNAPESCA Certification Programs.
3) The procedure must clearly state the roles and responsibilities of the staff involved. Identifying at least one analyst responsible for informing all the results and one Head of Laboratory, responsible for verifying that the non-compliant results are informed to SERNAPESCA.
4) The procedure must ensure that whenever these analysis results are found, they will be verified against SERNAPESCA's regulations.
5) The procedure must establish keeping a record of unfavorable results in the laboratory, which may be inspected by SERNAPESCA's staff.

All unfavorable results related to SERNAPESCA's programs (CER, QAP, HPB, BMSP, Phytoplankton, Residues, Contaminants) must be informed immediately to SERNAPESCA, through the Unfavorable Results Report (IRD) and sent to the National Directorate of SERNAPESCA and the interested party, via email. The IRD must be sent as an *Excel* spreadsheet provided by the person in charge of the Laboratories Program of the Foreign Trade Sub-Directorate.

In the specific case unfavorable results for parasites, these must be reported in the previously mentioned *Excel* spreadsheet form, indicating that it has been sent out for confirmation in the "Remarks/Notes" section.

For those laboratories that outsource analyses, these will be responsible for informing any unfavorable results found by the outsourced laboratory.

1.7 PROCEDURE FOR SOLVING CONTROVERSIES RELATED TO THE RESULTS OF CONTROL ANALYSES FOR PROHIBITED AND UNAUTHORIZED SUBSTANCES IN FARMS, FAR SERNAPESCA VERIFICATION, VERIFICATION AND SERNAPESCA VERIFICATION AND END PRODUCT CONTROL THROUGH A CERTIFICATION PROGRAM

This procedure applies both to the results analyses of pharmaceutical products residues, as well as to contaminants, unauthorized and prohibited substances in farm fish, issued by the Verification Laboratory of SERNAPESCA within the Residues Control Program of SERNAPESCA, as well as to the chemical analyses issued by the Verification Laboratories of SERNAPESCA and Service Laboratories for the control to issue the approval for the End Product through the Certification Program (SMAE) and according to the QAP Quality Assurance Program.

Its execution may be requested by any company that is part of the programs above, which disagrees with the results issued by the Laboratory, to make the most accurate decision for the affected products.

The Company may request the execution of the following procedures, considering the terms set in each stage: 1.- Review of analysis procedures, 2.- Inspection visit to the Laboratory and 3.- Sending counter samples to an international reference laboratory (FAR and QAP Official Verifications) or to a SERNAPESCA Verification Laboratory (QAP Verifications and End Product Control).

This request must be presented in writing to SERNAPESCA, clearly explaining the reasons why the Company disagrees with the Laboratory results.

1.7.1 REVIEWING ANALYSIS PROCEDURES

The Company may request the laboratory to carry out a special review of the records involved in the samples analyses. This request must be made to the laboratory via email, with a copy to the National Directorate of SERNAPESCA, within 3 business days from the issuance of the Results report. The Laboratory must write a report within 3 business days after receiving the request. The report must be sent from the laboratory to the company with a copy to the National Directorate of SERNAPESCA via email.

1.7.2 INSPECTION VISIT TO THE LABORATORY

The Company may request an inspection visit to the Laboratory to verify on site, the operation of the quality system and its technical competencies. The request must be sent via email to the Laboratory with a copy to the National Directorate of SERNAPESCA and to the Regional Directorate under whose jurisdiction the laboratory to be inspected is, within 3 days from the issuance of the Results report. It must explain the reasons why the visit is being requested and the purpose that it intends, propose dates for this and point out the people that will be part of the process and their corresponding positions. The laboratory must reply to the request within 3 business days, indicating if it accepts or does not accept the proposed dates and if there are any objections regarding the people that will be part of the visit. If the dates proposed by the Company are not accepted by the Laboratory, it must propose new dates trying to coordinate a day that is suitable for both parties.

This visit must be accompanied by a SERNAPESCA official of the laboratory's jurisdiction, who must leave a record of the visit to the laboratory in SERNAPESCA's book, with notes or comments.

It must also be added that the failure to coordinate the visit will lead to its suspension.

If after the visit, the company still has doubts, it must send a report to the laboratory with a copy to the corresponding National and Regional Directorate, with comments on the visit and well-founded reasons stating why it still does not agree with the results of the reported analysis. The laboratory will assess the content of the report and will reply to the comments. Based on the conclusions obtained, and if an agreement has not been reached by the parties, SERNAPESCA will determine the need to take any new actions for the analysis results informed.

1.7.3 SENDING COUNTER SAMPLES TO AN INTERNATIONAL REFERENCE LABORATORY OR A SERNAPESCA VERIFICATION LABORATORY

The purpose of this procedure is to dispel any reasonable doubts that may exist regarding the results delivered by a SERNAPESCA Verification Laboratory or Service Laboratory. In case of requesting the delivery of counter samples to an International Reference Laboratory (FAR and QAP

Official Verifications) or a SERNAPESCA Verification Laboratory (Biweekly QAP Verifications and End Product Control through the Certification Program), this procedure must be followed:

a) The counter sample delivery process will not take place until SERNAPESCA has the necessary guarantees that the product will not be distributed for consumption, during the controversy.
b) The delivery of counter samples must be requested in writing to the Foreign Trade Sub-Directorate, with a copy to the Offices of SERNAPESCA under whose jurisdiction the production facility is, as well as the laboratory in question. The request must be sent within 10 business days after the issuance of Laboratory analysis report and must state clearly the reason(s) why the procedure is being requested. The Foreign Trade-Sub Directorate must reply within 3 business days after receiving the request.
c) The counter samples will be sent to an International Reference Laboratory previously assigned by the Service, for the results provided by the SERNAPESCA Verification Laboratories coming from SERNAPESCA Verifications of the Pharmaceutical Residues and Quality Assurance Programs. On the other hand, the counter samples will be sent to a SERNAPESCA Verification Laboratory previously assigned by SERNAPESCA, for the results provided by private laboratories, coming from Verifications of the Quality Assurance Program and of the results coming from the End Product Control.
d) All official counter samples from the analysis report will be sent out for analysis. This is those counter samples collected by the SERNAPESCA Verification Laboratory or the Service Laboratory and stored in their facilities. Sending them together with other samples that do not correspond to those previously described will not be accepted.
e) The laboratory will be responsible for hiring the services of a *courier* service that will transport the counter samples, by mutual agreement with the company. Counter samples must be delivered within 20 business days from the issuance of the results report of the SERNAPESCA Verification Laboratory or the Service Laboratory. If the term has reached an end and if there are not any justified reasons, the procedure will be deemed as void.
f) The delivery of counter samples must take place in the presence of an official SERNAPESCA inspector and a representative of the company, due to which the procedure must be previously coordinated with the SERNAPESCA Office under whose jurisdiction the laboratory is located.
g) The counter samples must be properly packed, to withstand their transportation without any inconveniences. Each counter sample must be identified individually. In the case of frozen products, these must be packed with *gel packs* or dry ice to keep them in proper conditions until they are received at the destination laboratory.
h) The official tape of SERNAPESCA must be used to pack the counter samples.
i) The company must conduct all the proceedings with SERNAPESCA for the sanitary certification needed to transport counter samples, as stated in Section III of this Manual, for samples without commercial value and not fit for human consumption,
j) The National Directorate of SERNAPESCA will coordinate the reception of the samples with the International Reference Laboratory or the SERNAPESCA Verification Laboratory. This laboratory will then inform the results of the analyses directly to SERNAPESCA.
k) All the packaging, transportation and analysis costs involved in this procedure will be in charge of the requesting company. The company must provide the Foreign Trade Sub-Directorate its complete information for the International Reference Laboratory or the Verification Laboratory of SERNAPESCA to invoice the costs for the analyses.

1.7.4 REGARDING THE RESULTS OBTAINED FROM THE COUNTER SAMPLES ANALYSIS

The result obtained from the analysis of counter samples will be considered as final, whether it is greater or lower than the one initially reported.

For the purposes of the evaluation of the laboratory in question, if the difference between the result(s) of the analysis initially informed and the result(s) of the counter samples is greater than

the critical difference Δ^1 that would be expected according to the uncertainty of the measurements of both parties, the laboratory in question will be requested to conduct an internal investigation of the reasons and to adopt immediate corrective actions.

1.7.5 REGARDING THE SANITARY CERTIFICATION

If the product involved in the FAR and QAP Official Verifications, in the QAP Periodical Verifications or in the End Product Control, requires a sanitary certification from SERNAPESCA, this will not be provided until obtaining the final result from the International Reference Laboratory or the Verification Laboratory of SERNAPESCA.

If the sanitary certificate has been provided to the company, it must be immediately returned to SERNAPESCA, until having the final result from the International Reference Laboratory or from the Verification Laboratory of SERNAPESCA.

If the sanitary certificates are in destination with the product, the Company must guarantee that the product will not enter the country of destination or will not be distributed, until having a final result from the International Reference Laboratory or from the Verification Laboratory of SERNAPESCA.

1.8 PROCEDURES RELATIVE TO THE BIVALVE MOLLUSKS SANITATION PROGRAM (BMSP)

The authorized Laboratory must verify that the information included in the BMSP Sampling and Analysis Form (Part III, Annexes, Chapter) matches the received samples, and immediately enter it in the Mr-Sat (Red Tide - Early Alert System) computer system. Similarly, it must make sure that the form is duly stamped by the SERNAPESCA office at origin and that the samples are in proper conditions to start the corresponding analyses.

If the form is not stamped and/or the samples have not been received within the required timeframe, and in proper condition, the entity must enter the cause for rejection in the Mr-Sat system and inform the situation to SERNAPESCA and the interested party, to conduct a new sampling if deemed necessary. If the sample is in suitable conditions to conduct the analysis but does not comply with the timeframe set and/or the form is not stamped, the laboratory must immediately inform the National Directorate, who will determine if the sample is to be accepted or rejected.

In case of rejection of the sample, for administrative reasons, the interested party will have a 48 hours period to rectify the situation with the laboratory, otherwise, the rejection will become effective. The above must be informed immediately to the National Directorate, with a copy to the corresponding Regional Directorate.

Once the sample is received, the proceedings indicated in the form above must be undertaken, through the official SERNAPESCA analysis methodologies for the BMSP.

The determination of marine biotoxins must be undertaken by the authorized laboratories, using the analysis methodologies described in Chapter III, Item 3.

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[1] $\Delta = \sqrt{U_1^2 + U_2^2}$ donde U_1 y U_2 son las incertidumbres de la medición ampliada de los resultados de ambas partes

Once the analyses have been conducted, the entity must enter the results into the Mr-Sat (Red Tide - Early Alert System) computer system, within 12 hours from obtaining the results. In parallel, the entity will issue the corresponding analysis report, which must be sent to the National Directorate together with a copy of the BMSP Sampling and Analysis Form, in electronic format (pdf), within 48 hours of obtaining the results. A copy of these reports must be sent by the analysis entity to the company in charge of monitoring the area.

If there are any unfavorable results in the analyses or if they fall outside of the parameters set by SERNAPESCA in terms of products, these must be immediately reported (within the next 2 hours) to the National Directorate of the Service, to the corresponding Regional SERNAPESCA office and the interested party, as set forth in Item 1.6 of this Chapter. The National Directorate will inform the certifying regions.

1.8.1 COMPLIANCE WITH THE REQUIREMENTS SET FORTH

The samplers and analysis entities must comply with all the requirements set forth. If there are any non-compliances with these requirements, either from the analysis laboratories or the samplers, sanctions will be applied, which go from a temporary to an indefinite suspension from SERNAPESCA's system.

These non-compliances subject to sanctions for samplers are the following, among others:
- Delays in sending the samples to the analysis laboratory.
- Non-compliance with the monitoring program.
- Attending a sampling without the necessary equipment.
- Sending samples to unauthorized laboratories.

These non-compliances subject to sanctions for analysis entities are the following, among others:
- Delays in sending the results of the analyses.
- Non-compliances in the unfavorable results report.

1.8.2 CONTROL

The official of the Foreign Trade Sub-Directorate must verify the aspects of the fisheries regulation, as informed by the Department of Control of the corresponding region.

Any new measures must be informed by the Department of Control, in writing, if possible, including their purpose, start and end date and any other details needed for its application.

2. PHYSICAL-CHEMICAL ANALYSIS METHODS FOR EXPORT FISHERY PRODUCTS

The following section describes the official technical standards based on which laboratories must implement the physical-chemical analysis methods for export fishery products.

Alternative methods will be accepted, to the extent that they are duly verified and validated, as appropriate.

2.1. DETERMINATION OF pH

a) Scope:
This method applies to fishery products in general.
b) Method:
NCh 2738 Of2002: Hydrobiological Products - Measurement of pH.
c) Reference:
- ISO 2917 Meat and meat products – Measurement of pH – Reference method, second edition.
- AOAC Official Method 964.24, 1995, Buffer Solutions for Calibration of pH Equipment.

2.2. DETERMINATION OF MOISTURE CONTENT

a) Scope:
This method applies to fishery products in general.
b) Method:
NCh 2670.Of2001: Hydrobiological Products - Determination of Moisture Content.
c) Reference:
- International Standard ISO 1442:1997 Meat and meat products – Determination of moisture content (Reference methods).
- AOAC, Official Method 937.07, 1996 Fish and marine products - Treatment and Preparation of Sample Procedure.
- AOAC, Official Method Ca 2a-45, 1973, Method Distillation Method.
- Chilean Standard NCh 512.Of80. Fish meal. Determination of Moisture Content.

2.3. DETERMINATION OF CHLORIDE AS SODIUM CHLORIDE (I), VOLHARD METHOD

a) Scope:
This method applies to fishery products in general, except for salted or dried salted fishery products.
b) Method:
NCh 2739/1.Of2002: Hydrobiological Products - Determination of chlorine - Part 1 - Volhard Method.
c) Reference:
- AOAC Official Method 937.09 Salt (chlorine as Sodium Chloride) in seafood Volumetric Method. Final Action.
- AOAC Official Method 937.07 Fish and Marine Products. Treatment and Preparation of Sample. Procedure. Final Action 1996.

2.4. DETERMINATION OF CHLORIDE AS SODIUM CHLORIDE (II), MOHR METHOD

a) Scope:
This method applies to dry and dried salted fishery products.
b) Method:
NCh2739/2.Of2002: Hydrobiological Products - Determination of chloride - Part 2 - Mohr Method.
c) Reference:
- Codex Standard for Salted Fish and Dried Salted Fish of the Gadidae of Fishes: Codex Stan 167-1989, Rev. 1-1995.
- AOAC Official Methods 937.07, 1976, Fish and Marine Products - Treatment and Preparation of Sample. Procedure.

2.5. DETERMINATION OF TVBN

a) Scope:
This method applies to fishery products in general.
b) Method:
NCh 2668.Of2001: Hydrobiological Products - Determination of Total Volatile Basic Nitrogen (TVBN).
Note: The method of distillation of an extract deproteinized by trichloroacetic acid must be employed.
c) Reference:
- CODEX ALIMENTARIUS, 1986, 14/8 Manuals of Food Quality Control, Total Volatile Bases in Fish.
- AOAC, Official Methods 937.07, 1996, Fish and Marine Products - Preliminary Treatment and Preparation of Sample.

2.6. DETERMINATION OF TRIMETHYLAMINE NITROGEN

a) Scope:
This method applies to raw and dried salted fishery products.
b) Method:
NCh 2757.Of2002: Hydrobiological Products - Determination of Trimethylamine Nitrogen.
c) Reference:
- AOAC, Official Method 35.1.17 (971.14), 2000 Trimethylamine nitrogen in seafood – Colorimetric method.
- AOAC, Official Method 35.1.01 (937.07), 2000 Fish and Marine Products – Treatment and Preparation of Sample – Procedure.

2.7. DETERMINATION OF HISTAMINE

a) Scope:
This method applies to frozen, canned, smoked, breaded, dried, salted and dried salted fishery products.
b) Method:
The sample is extracted with methanol. The extract obtained is passed through an ion exchange column. The eluate is treated with o-Phthalaldehyde to form fluorescent histamine derivatives. The fluorescent intensity of the derivative is measured by a fluorometer, and the histamine is quantified using external standards.

- Reagents:
  - Hydrochloric Acid, HCl, 37%, p.a
  - Sodium Hydroxide, NaOH, p.a.
  - Phosphoric Acid, H_3PO_4, 85%, p.a.
  - O-Phthalaldehyde (OPT) p.a.
  - Histamine Dichlorohydrate, $C_5H_{11}Cl_2N_3$, 98% minimum.
  - Methanol, CH_40 p.a.
  - Water for analysis, Class 1, as per NCh426/2.
  - Basic ion-exchange resin, strong anion. Dowex 1-X8, 50-100 mesh or equivalent.
  - Potassium hydrogen phthalate, $C_8H_5KO_4$, p.a.
  - Phenolphthalein, $C_20H_{14}O_4$, p.indicator.

- Solutions:
  - Hydrochloric Acid HCl 1N: Measure 83ml of HCl, 37%, p.a. and carefully add them over approximately 200ml of water for analysis, cool, dilute at 1L and shake.

- Hydrochloric Acid HCl 0.1N: Measure 8.3ml of HCl, 37%, p.a. and carefully add them over approximately 200ml of water for analysis, cool, dilute at 1L and shake.
- Sodium Hydroxide NaOH 1N: Weigh 40g of NaOH in water for analysis and dilute at 1L. Standardize with Potassium hydrogen phthalate.
- Sodium Hydroxide NaOH 2N: Weigh 40g of NaOH in water for analysis and dilute at 500ml.
- Standardized 3.57N Phosphoric Acid: Dilute 121.8ml of H3P04 p.a. at 1L with water for analysis. Standardize 5.00ml with NaOH 1N using phenolphthalein as an indicator. Adjust the concentration if needed.
- 1mg/ml solution of pattern of histamine: Weigh 169.10mg of Histamine Dichlorohydrate (98%), dissolve and dilute with HCl 0.1N. Prepare this solution on a weekly basis and keep in the refrigerator.
- 10μg/ml intermediate histamine solution: Pipet 1ml of solution of pattern of histamine in a 100ml graduated flask and dissolve with HCl 0.1N. Prepare this solution on a weekly basis.
- 0.5; 1.0; and 1.5μg/5ml of histamine work solutions: Pipet 1, 2 and 3ml of the intermediate solution in separate 100ml graduated flasks and dissolve with HCl 0.1N. Prepare these solutions on a weekly basis.
- 75% Methanol: Place 75% methanol (distilled in glass) in a 100 ml graduated flask or in a 100ml test tube. Dilute at 100ml with water for analysis. Shake the jar gently while adding water.
- 0.1% O-Phthalaldehyde (OPT): Dissolve 100mg of OPT in 100mg of methanol (distilled in glass). Store in an amber glass bottle in the refrigerator. Prepare fresh solution on a weekly basis.
- OH-form conditioned Ion-exchange resin

- Preparation of the resin:
  - Place the resin in a glass and add 15mL of NaOH 2N per gram of resin.
  - Mix gently and decant for 20 minutes, and no more than 30 minutes.
  - Decant the liquid.
  - Repeat the procedure with an additional amount of soda.
  - Wash the resin carefully with water for analysis, soak with absorbent tissue and wash again with water for analysis, as many times as needed until proving a neutral pH with the dipstick.
  - Prepare fresh resin on a weekly basis and store it covered with water for analysis.

- Preparation of the column:
  - Place glass wool in the bottom of the column and add prepared resin until forming a bed of 8cm. Keep the water above the upper level of the resin bed. Do not regenerate the resin in the column, it must be done in a glass as needed.
  - Wash the column with 10mL of water for analysis before adding each extract.

- Tools:
  - Fluorometer equipped for exciting conditions at 350 nm and emission at 444 nm.
  - 200 x 7 mm polypropylene column (inner diameter) with a small plastic stopcock and approximately 45 cm of Teflon tube. Alternatively, a 2-way stopcock can be used instead of a Teflon tube.

- Procedure:
  - Preparation of the sample:
  - Canned products: After opening the container, the liquid covering it must be drained, and then the homogenate of the drained product must be prepared in a food processor.

- Fishery or frozen products: Thaw if needed, discard bones and skin, and then chop. Prepare the product's homogenate in a food processor.
- Salted Fishery Products: Prepare brine with regular salt and immerse the salting product for some seconds to remove the salt from the surface. Take the product out of the solution and dry with absorbent paper, remove bones, chop and prepare its homogenate in the processor.
- Dried salted, breaded or smoked fishery products: Homogenize the sample in a food processor.

- Treatment of the sample:
  - Weigh 2.5g ± 0.5g of dried product or ± 10g of fresh or canned seafood, in a *blender* cup at high speed. Record the weight (M).
  - Add 30 mL of methanol and place in the *blender* for 2 minutes.
  - Pour into a 50 mL or 100 mL volumetric flask and rinse the *blender* with methanol.
  - Heat in a water bath for 15 minutes at 60 C°; and cool at room temperature, gage with methanol and mix.
  - Filter with folded filter paper or centrifuge at approximately 3600 rpm for 5 minutes. This methanolic filtrate can be stored refrigerated for up to approximately 1 month; (a light layer of powder could accumulate in the bottom during storage, which does not interfere with the analysis).
  - Pass 45 ml of water through the previously prepared column, and remove the eluate.
  - Place a 50 mL volumetric flask, which contains 5 mL of HCl 1 N in the outlet of the column.
  - Pipet 1 mL of the methanolic extract in the column and add between 4 and 5 mL of water, and start eluting, once the volume is near the limit of the resin, continue adding water until almost reaching the measurement of the flask.
  - Receive the eluate in the 50 mL volumetric flask that contains 5 mL of HCl 1N.
  - When the level of the liquid reaches approximately 2 mm in the column, over the resin, add 5 mL of water and continue eluting.
  - Continue adding distilled water under these conditions until completing 50 mL of eluate and mix. Refrigerate the eluate if not continuing with the determination immediately.

- Measurements:
  - Pipet 5 mL of each one of the solutions of the samples and of the standards in a 50 mL glass or polypropylene *Erlenmeyer* flask, separately.
  - Add 10 mL of HCl 0.1 N in each *Erlenmeyer* flask and mix.
  - Add 3 mL of NaOH 1N in each *Erlenmeyer* flask and mix.
  - Add 1 mL of OPT within the next 5 minutes in each *Erlenmeyer* flask and mix immediately.
  - Exactly 4 minutes after adding the OPT, add 3 mL of H_3PO_4 3,75 N and mix immediately; it is important to mix thoroughly after adding.
  - Conduct from 6 to 10 reactions with OPT simultaneously, adding the reagents to the *Erlenmeyer* flask, in the sequence described.
  - Prepare a blank with 5 mL of HCl 0.1N instead of the problem solution.
  - After 1.5 hours, read the fluorescence intensity of the standards, using water in the reference cell after 30 minutes as a minimum and 1.5 hours as a maximum, using an excitation wavelength of 350 nm and an emission wavelength of 444 nm. Plot the intensity values (corrected by the blank) against μg of histamine in 5 mL aliquot.
  - Read the fluorescence intensity of the samples in the conditions above and calculate the histamine concentration in the sample.
  - If the histamine content were too high, repeat the reaction with a dilution made with HCl 0.1 N, repeating the measurement process from the beginning.

- Expression of results:

$$Histamine,\ ppm = \frac{(\mu\ histamine/5mL\ obtained\ from\ the\ curve) \times F}{M}$$

Where:

$$F\ (Dilution\ factor) = \frac{Measurement\ Volume\ 1 \times Measurement\ Volume\ 2}{Aliquot\ 1 \times Aliquot\ 2}$$

Where:
- Measurement 1: Sample preparation initial measurement volume.
- Aliquot 1: Aliquot introduced in the column.
- Measurement 2: Eluate measurement volume.
- Aliquot 2: Volume taken for reaction.

c) Reference:
AOAC Official Methods 35, 1, 32 (977,13) modified in March, 1997.

2.8. DETERMINATION OF HISTAMINE BY HPLC

a) Scope:
The method applies to fresh and frozen fishery products and fish meal.
b) Method:
NCh 2637.Of2001: Hydrobiological products - Determination of histamine and other biogenic amines - HPLC method with UV detector.
c) Reference:
- AOAC, Official Methods 937.07, 1976, Fish and Marine Products – Treatment and Preparation of Sample – Procedure.
- Food, Water, and Soil Physical-Chemical Analyses Manual, 1998, Public Health Institute: Determination of Histamine.
- JOURNAL OF CHROMATOGRAPHY.
  - 107(1975)416-419, Separation of Dns Derivates of Polyamines and Related Compounds by Thin-Layer and High-Pressure Liquid Chromatography.
  - 124(1976)277-285, Determination of Diamines and Polyamines in Tissues by High-Pressure Liquid Chromatography.
  - 145(1978)221-229, Sensitive Fluorimetric Method for the Determination of Putrescine, Spermidine, and Spermine by High-Performance Liquid Chromatography and its Application to Human Blood.

2.9. DETERMINATION OF MERCURY

a) Scope:
This method applies to canned, frozen, salted, and dried fishery products; fresh-refrigerated fish and fresh-refrigerated and/or live mollusks.
b) Method:
NCh 2667.Of2001: Hydrobiological Products – Determination of mercury – Cold Vapor Atomic Absorption Spectrophotometric Method.
c) Reference:

AOAC Official Methods, 2000; 9.2.23 (977.15) Mercury in Fish. Alternative Flameless Atomic Absorption Spectrophotometric Method.

2.10. DETERMINATION OF LEAD

a) Scope:
Determination of fishery products in general.
b) Method:
NCh 2751.Of2003: Hydrobiological Products – Determination of lead by atomic absorption spectrophotometry.
c) Reference:
- AOAC Official Methods, 2000; 9.2.17 (972.23) Lead in Fish. Atomic Absorption Spectrophotometric Method.
- AOAC, Official Methods, 2000; 35.1.01 (937.07), Fish and Marine Products Treatment and Preparation of Sample – Procedure.

2.11. DETERMINATION OF ARSENIC

a) Scope:
This methodology applies to fishery products in general.
b) Method:
NCh 3140. Of2008: Hydrobiological Products - Determination of arsenic by hydride generation atomic absorption spectrophotometry.
c) Reference:
- AOAC Official Methods 986.15 Arsenic, Cadmium, Lead, Selenium, and Zinc in Human and Pet Foods, version 2005.

2.12. DETERMINATION OF TIN

a) Scope:
Determination of canned fishery products.
b) Method:
NCh 2761.Of 2005 - Canned food - Determination of tin by flame atomic absorption spectrophotometry.
c) Reference:
NCh 2761.Of 2005 - Canned food - Determination of tin by flame atomic absorption spectrophotometry.

2.13. DETERMINATION OF ZINC

a) Scope:
This methodology applies to fishery products in general.
b) Method:
AOAC Official Methods 986.15 Arsenic, Cadmium, Lead, Zinc. Multi-element First Action Method 1990, Final Action 1992.
c) Reference:
AOAC Official Methods 986.15 Arsenic, Cadmium, Lead, Zinc. Multi-element First Action Method 1990, Final Action 1992.

2.14. DETERMINATION OF CADMIUM

a) Scope:
This methodology applies to fresh or processed fishery products and fish meal. In the case of scallops corresponding to the Bivalve Mollusks Sanitation Program, the analysis is conducted in the muscle and gonad.
b) Method:
NCh 2638.Of2001: Hydrobiological Products – Determination of cadmium – Flame atomic absorption spectrophotometric method.
c) References:
- AOAC Official Methods 969.32 Zinc in Food. Atomic Absorption Spectrophotometric Method. First Action 1969. Final Action 1971.
- AOAC Official Methods 937.07, 1976, Fish and Marine Products – Treatment and Preparation of Sample – Procedure.
- NCh 2313/10.Of96 Waste Water – Analysis Methods – Part 10: Determination of Heavy Metals – Flame Atomic Absorption Spectrophotometry Method.
- Food, Water, and Soil Physical-Chemical Analyses Manual, 1998, Public Health Institute:

2.15. DETERMINATION OF CHROMIUM

a) Scope:
Methodology applicable to fish meal.
b) Method:
Determination of chromium by atomic absorption.

2.16. DETERMINATION OF MELAMINE

a) Scope:
Methodology applicable to fish meal.
b) Method:
Determination of melamine by liquid chromatography with visible UV detector or a method with similar or better analytic characteristics.

2.17. DETERMINATION OF BENZOPYRENE

a) Scope:
This methodology applies to bivalve mollusks, smoked and non-smoked fish flesh, non-smoked crustaceans and cephalopods, and oils.
b) Method:
Determination of benzopyrenes by HPLC-Fluorescence.
c) References:
- Enrichment of benzo[a]pyrene in smoked food products and determination by high-performance liquid chromatography-fluorescence detection. Journal of Chromatography A, Volume 753, Issue 2, 15 November 1996, Pages 207-215. M.S. García Falcón, S. González Amigo, M.A. Lage Yusty, M.J. López de Alda Villaizán, J. Simal Lozano.
- Assessment of Polycyclic Aromatic Hydrocarbon Content of Smoked Fish by Means of a Fast HPLC/HPLC Method, Sabrina Moret, Lanfranco Conte, and Daniela Dean J. Agric. Food Chem., 1999, 47 (4), pp 1367–1371.
- Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection Original Research Article Journal of Chromatography A, Volume 1217,

Issue 35, 27 August 2010, Pages 5555-5563 A. Ishizaki, K. Saito, N. Hanioka, S. Narimatsu, H. Kataoka.

2.18. DETERMINATION OF MOISTURE CONTENT AND IMPURITIES IN SEAWEEDS

a) Scope:
This methodology applies to dry seaweeds.
b) Method:
NCh 765.Of2002: Dry seaweeds - Determination of moisture content and impurities.
c) Reference:
Chilean Standard NCh 765. DRY SEAWEEDS. Determination of Moisture Content and Impurities.

2.19. DETERMINATION OF ANIMAL ORIGIN COMPONENTS IN MEAL

a) Scope:
This methodology applies to fish meal.
b) Method:
COMMISSION REGULATION (EC) 152/2009, Annex VI - Analysis methods to determine animal origin components for the official control of feed.
c) Reference:
COMMISSION REGULATION (EC) 152/2009, Annex VI - Analysis methods to determine animal origin components for the official control of feed.

2.20. CHEMICAL METHODS FOR POTABLE WATER ANALYSIS

The characteristics of water analyses required by the European Union are detailed as follows.

Table: *Characteristics of water analyses required by the European Union*

PARAMETERS	UNIT	ACCURACY	PRECISION	Detection Limit
		% of the parametric value	% of the parametric value	% of the parametric value
Aluminum	ug/l	10	10	10
Conductivity	uS/cm at 20°C	10	10	10
Oxidability	mg/l O2	25	25	10
Sodium	mg/l	10	10	10
1.2-dichloroethane	ug/l	25	25	10
Antimony	ug/l	25	25	25
Benzo(a)pyrene	ug/l	25	25	25
Boron	mg/l	10	10	10
Bromate	ug/l	25	25	25
Polycyclic aromatic hydrocarbons	ug/l	25	25	25
Nickel	ug/l	10	10	10
Tetrachloroethene and trichloroethene	ug/l	25	25	10

2.21. GUIDELINES FOR VERIFYING THE ABSENCE OF PARASITES IN FISHERY PRODUCTS

The purpose of this procedure is to verify the absence of internal parasites, such as Cestodes (Diphyllobothrium), Nematodes (Anisakis) in export fishery products.

The organoleptic physical analysis currently conducted in QAP verification plants and in the Certification of fresh, refrigerated and frozen fishery products will be complementary to the procedure outlined in Item 2.18.a of this Chapter.

2.21.1. ANALYSIS PROCEDURE

- If samples of whole or eviscerated, headless and tailless fish are received, the laboratory will conduct a visual, non-destructive examination to the viscera and/or visceral walls of the muscles.
- In the case of receiving fish samples, in any presentation, coming from farms or fish samples coming from extractive fishing in different presentations from those mentioned in Item 2.1.35, 200g of muscle pieces will be taken for visual examination. The samples must be clean and without skin. Both sides of the muscle will be examined (filet) under a direct source of light and with the help of a magnifying glass. Optionally, both sides of the muscle (filet) may be examined with UV light with a wavelength of 366 nm in a dark room. Afterward, 10 thin filets will be obtained for each sample, no thicker than 4 mm, to be checked under the *Candling* system.
- In the absence of parasites, "absence of visible parasites" will be informed.
- In the presence of parasites, their genus and, if possible, their species must be confirmed. For that, any suspicious samples must be fixed for their further delivery to a laboratory authorized by SERNAPESCA for their confirmation. Parasite fixation will be done in FAA (10 parts of 37 – 40% formalin, 70 parts of 95% ethanol, 15 parts of water and 5 parts of acetic acid, FDA), or in a formalin-saline solution (4 parts of formalin and 96 parts of 1% sodium chloride).

2.21.2. AUTHORIZATIONS

- Those Service Laboratories authorized under the framework of the SERNAPESCA Certification System to conduct the organoleptic physical examination of fishery products must conduct the analysis to determine the presence of parasites according to the procedure described in these guidelines. In addition, they must confirm before this Service, that the analyst in charge of conducting such analysis is duly trained for this purpose.
- Those university laboratories authorized by SERNAPESCA to conduct Official Verifications of the Quality Assurance Programs of the plants with QAP must also comply with the previous item.
- Those plant laboratories with QAPs authorized by SERNAPESCA to conduct biweekly Verifications of the Quality Assurance Programs may include this analysis in the scope of their authorization, to the extent that they comply with Chapter I, Item 1.
- Any laboratory that wishes to conduct confirmation analyses must request it in writing to the National Directorate of SERNAPESCA. To be authorized, the laboratory must prove to have a vast experience in the study of aquatic parasites with an emphasis in conducting research in this area. The laboratory must be directed by a professional in the area of biology or chemistry, with at least 5 years of experience in the area and 1 year of post-graduate studies in aquatic species parasites.

2.22. PEROXIDE VALUE

a) Scope:

This methodology is applied to raw, semi-refined, refined, winterized and acidulated fish oil, as well as to edible oil coming from canned hydrobiological products.
b) Method:
NCh 2758.Of2002: Fish oil - Determination of peroxide value.
c) Reference:
NCh 2758.Of2002: Fish oil - Determination of peroxide value.

2.23. FREE FATTY ACIDS

a) Scope:
This methodology applies to the content of free fatty acids presents in raw, semi-refined, refined, winterized and acidulated fish oil.
b) Method:
NCh 2759.Of2002: Fish oil - Determination of free fatty acids.
c) Reference:
NCh 2759.Of2002: Fish oil - Determination of free fatty acids.

2.24. DIOXINS (PCDD/PCDF) and PCBs

a) Scope:
This method is used to determine the content of Dioxins and dioxin-like PCBs in hydrobiological products and fish meal and oil.
b) Method:
Determination of dioxins (PCDD/PCDF) and dioxin-like PCBs by High-Resolution Gas Chromatography and High-Resolution Mass Detector (HRGC-HRMS).
c) Reference:

- EPA, 1994.Method 1613. Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC-HRMS, Revision B. 86 pp.
- EPA, 2008.Method 1668B.Chlorinated Biphenyl Congeners in Waters, Soil, Sediment, Biosolids, and Tissue by HRGC-HRMS.128 pp.
- EPA 2007, Method 3545A, Pressurized fluid extraction (PFE), Revision 1. 16 pp.
- Haglund, P.; Korytar, P., Danielsson, C.; Díaz, J.; Wiberg, K.; Leonards, P.; Brinkman, U.A.T.; Boer, J.; GCxGC-ECD: a promising method for the determination of dioxins and dioxin-like PCBs in food and feed, Anal. Bioanal. Chem. 390 (2008) 1815-1827.
- Ortiz, X.; Gasser, M.; Marti, R.; Montaña, M.J.; Margarit, L.; Broto, F.; Díaz-Ferrero, J.; Fractionation of persistent organic pollutants in fish oil by high-performance liquid chromatography equipped with a 2-(1-pyrenyl)ethyl silica column, OrganohalogenCompds. 70 (2008) 2416-2419.
- Marti, M.; Ortiz, X.; Gasser, M.; Marti, R.; Montaña, M.J.; Díaz-Ferrero, J.; Determination of POPs (PCDD/F, dioxin-like PCBs, marker PCBs, PBDEs and OCP) in health supplements on the Spanish market.
- European Union, 2002. Commission Directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs.
- European Union, 2006. COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 20 pp.
- European Union, 2006. COMMISSION REGULATION (EC) No 199/2006 of 3 February 2006 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs. 5 pp.
- European Union, 2006. COMMISSION DIRECTIVE 2006/13/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs. 10 pp.

- European Union, 2011. COMMISSION REGULATION (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs. 6 pp.
- Van den Berg, et al., 2005. REVIEW: The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalent Factors for Dioxins and Dioxin-Like Compounds. Toxicological Sciences 93(2), 223-241.pp
- European Union, 2012. COMMISSION REGULATION (EU) No 277/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls.
- European Union, 2012. COMMISSION REGULATION (EU) No 594/2012 of 5 July 2012 amending Regulation (EC) 1881/2006 as regards the maximum levels of the contaminants ochratoxin A, non-dioxin-like PCBs and melamine in foodstuff, as regards the maximum levels of the contaminants ochratoxin A, non-dioxin-like PCBs and melamine in foodstuff.
- European Union, 2013. COMMISSION REGULATION (EU) No 1067/2013 of 30 October 2013 amending Regulation (EC) No 1881/2006 as regards maximum levels of the contaminants dioxins, dioxin-like PCBs and non-dioxin-like PCBs in the liver of terrestrial animals.
- European Union, 2014. COMMISSION REGULATION (EU) No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls.
- European Union, 2014. COMMISSION REGULATION (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012.

2.25. NITROSAMINES (N-DIETHYLNITROSAMINE-NDEA AND N-DIETHYLNITROSAMINE-NDMA)

a) Scope:

This method is used to determine the content of Nitrosamines (n-Diethylnitrosamine-NDEA and n-Diethylnitrosamine-NDMA) in hydrobiological products (excluding fish oil).

b) Method:

Determination of Nitrosamines (n-Diethylnitrosamine-NDEA and n-Diethylnitrosamine-NDMA) by High-Performance Liquid Chromatography with mass detector in tandem (HPLC-MS/MS).

c) Reference:

- Wanfeng Wang, et al., 2010, Determination of N-nitrosodimethylamine in drinking water by UPLC-MS/MS, Journal of Environmental Sciences 22 (10) 1508-1512.
- Jeffrey W. A. Charrois, et al., Detecting N-Nitrosamines in Drinking Water at Nanogram per Liter Levels Using Ammonia Positive Chemical Ionization, Environmental Science & Technology 38, 4835-4841.
- Megan H. Plumlee, et al., N-nitrosodimethylamine (NDMA) removal by reverse osmosis and UV treatment and analysis via LC–MS/MS, Water Research 42 (2008) 347–355.
- Jong-eun Park, et al., Distribution of Seven N-Nitrosamines in Food, Toxicol. Res. Vol. 31, No. 3, pp. 279-288 (2015).

3. MARINE BIOTOXINS ANALYSIS METHODS IN EXPORT MOLLUSKS

The following section includes the references to the official methods recognized by SERNAPESCA, to conduct marine biotoxins analyses in export fishery products.

3.1. ANALYSIS METHODS

The following analyses must be conducted in the entire body or in the part intended for human consumption, both for the BMSP, as well as for the finished products control program, such as QAP and CER.

3.1.1. Paralytic Shellfish Poison (PSP)

a) Biological method:
- Reference:

AOAC Official Methods of Analysis, 18th Edition, 2005. Chapter 49:79-81 esc. 959.08.
Food and Drug Administration, Shellfish Laboratory Evaluation Checklist, 1994.

3.1.2. Toxins from the Group of Diarrhetic and Lipophilic Toxins

a) Biological method:
- Reference:

EU harmonized method. Version 4.0, April 2007 (Based on Yasumoto et al., 1984).

b) Instrumental method:
- Reference:

EU-Harmonized standard operating procedure for the determination of lipophilic biotoxins in mollusks by LC-MS/MS. European Union Reference Laboratory for Marine Biotoxins. Version - 5 June 2015.

High-performance liquid chromatography (HPLC) can be used as an alternative method with fluorometric detection or functional and immunological assays, such as the phosphate inhibition assay, to the extent that either separately or combined, they are at least as effective as the reference method, so that their application provides an equivalent level of public health protection, and that can detect at least the following analogs:

- Okadaic acid (OA) and dinophysistoxins (DTX1, DTX2 and any acetylated ester of OA and/or DTXs)
- Pectenotoxins: PTX1 and PTX2
- Yessotoxins: YTX, 45 OH YTX, Homo YTX and 45 OH Homo YTX,
- Azaspiracids: AZA1, AZA2 y AZA3.

If new important analogs related to public health are found, they must be included in the analyses. However, there must be patterns available, before conducting chemical analyses. Total toxicity will be calculated through conversion factors based on the data about the toxicity of each toxin.

3.1.3. Amnesic Shellfish Poison (ASP)

a) HPLC Method: Methanol-water extraction
b) Reference:

Quilliam, M.: Journal of AOAC International, Vol. 78, N°2, 1995.

4. ANALYSIS METHODS OF PHARMACEUTICAL PRODUCT RESIDUES AND CONTAMINANTS FOR EXPORT FISHERY PRODUCTS

The following section includes the list of monitored substances and the requirements and procedures set forth by SERNAPESCA in the Pharmaceutical Products Control Program, as well as references from the methods for conducting the pharmaceutical products residues and contaminants analysis in export fishery products.

4.1. LIST OF MONITORED SUBSTANCES

Below is a description of the monitored substances, the analysis methods and their Detection Limits (DL) and Quantification Limits (QL), valid for the SERNAPESCA Verification Laboratory: Veterinary Pharmacology Laboratory - Universidad de Chile

Table: *Analysis Methods and their Detection Limits (DL) and Quantification Limits (QL)*

Analysis	Matrix	Method	DL (ppb)	QL (ppb)
17 b estradiol	Salmon muscle and skin	GC MS/MS	1	2
2,4-D	Salmon muscle and skin	HPLC MS/MS	20	25
4-epi-Chlortetracycline	Salmon muscle and skin	HPLC MS/MS	5	6.3
4-epi-Oxytetracycline	Salmon muscle and skin	HPLC MS/MS	5	6
4-epi-Tetracycline	Salmon muscle and skin	HPLC MS/MS	5	5.3
Oxolinic acid	Salmon muscle and skin	HPLC MS/MS	5	5.1
Aldrin	Salmon muscle and skin	GC ECD	12	15
Amoxicillin	Salmon muscle and skin	HPLC FI.	5	10
Ampicillin	Salmon muscle and skin	LC MS/MS	20	25
Benzylpenicillin	Salmon muscle and skin	LC MS/MS	20	25
Cypermethrin	Salmon muscle and skin	LC MS/MS	0.5	0.53
Ciprofloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.2
Chloramphenicol	Salmon muscle and skin	HPLC MS/MS	0.1	0.2
Chlordane	Salmon muscle and skin	GC ECD	12	15
Chlortetracycline	Salmon muscle and skin	HPLC MS/MS	5	5.7
Colistin	Salmon muscle and skin	LC-MS/MS	50	60
Crystal violet	Salmon muscle and skin	HPLC MS/MS	0.1	0.2
Danofloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.1

Analysis	Matrix	Method	DL (ppb)	QL (ppb)
DDE	Salmon muscle and skin	GC ECD	12	15
DDT	Salmon muscle and skin	GC ECD	12	15
Deltamethrin	Salmon muscle and skin	LC MS/MS	0.5	0.55
Dichlorvos	Salmon muscle and skin	GC ECD	20	25
Dieldrin	Salmon muscle and skin	GC ECD	12	15
Dienestrol	Salmon muscle and skin	GC MS/MS	1	2
Diethylstilbestrol	Salmon muscle and skin	GC MS/MS	1	2
Diflubenzuron	Salmon muscle and skin	HPLC DAD	10	15
Diquat	Salmon muscle and skin	HPLC DAD	30	50
Emamectin	Salmon muscle and skin	HPLC Fl.	0.5	1.0
Enrofloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.3
Erythromycin	Salmon muscle and skin	HPLC MS/MS	0.5	1.5
Spectinomycin	Salmon muscle and skin	HPLC MS/MS	150	157.6
Steroids and Stilbenes	Salmon muscle and skin	GC MS/MS	1	2
Flavophospholipol	Salmon muscle and skin	HPLC MS/MS	300	320
Florfenicol	Salmon muscle and skin	HPLC UV/DAD	30	50
Flumequine	Salmon muscle and skin	HPLC MS/MS	5	5.2
Heptachloride	Salmon muscle and skin	GC ECD	12	15
Epoxy Heptachloride	Salmon muscle and skin	GC ECD	12	15
Hexestrol	Salmon muscle and skin	GC MS/MS	1	2
Leucocrystal violet	Salmon muscle and skin	HPLC MS/MS	0.1	0.2
Leucomalachite green	Salmon muscle and skin	HPLC MS/MS	0.1	0.2
Lincomycin	Salmon muscle and skin	HPLC DAD	40	42
Mirex	Salmon muscle and skin	GC ECD	12	15
Neomycin	Salmon muscle and skin	HPLC MS/MS	250	262.1
Nitrofurans (metabolites)	Salmon muscle and skin	HPLC MS/MS	0.3	0.5
Nitroimidazoles	Salmon muscle and skin	HPLC MS/MS	1	2

Analysis	Matrix	Method	DL (ppb)	QL (ppb)
Norfloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.2
Ofloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.4
Organochlorines	Salmon muscle and skin	GC ECD	12	15
Oxytetracycline	Salmon muscle and skin	HPLC MS/MS	5	5.7
Pefloxacin	Salmon muscle and skin	HPLC MS/MS	5	5.2
Sulphas (7)	Salmon muscle and skin	HPLC FI.	5	17
TDE	Salmon muscle and skin	GC ECD	12	15
Teflubenzuron	Salmon muscle and skin	HPLC DAD	10	15
Tetracycline	Salmon muscle and skin	HPLC MS/MS	5	5.7
Tilmicosin	Salmon muscle and skin	HPLC MS/MS	10	10.8
Tylosin	Salmon muscle and skin	HPLC MS/MS	10	12
Trimethoprim	Salmon muscle and skin	HPLC MS/MS	5	5.5
Brilliant green	Salmon muscle and skin	HPLC MS/MS	0.1	0.2
Malachite green	Salmon muscle and skin	HPLC MS/MS	0.1	0.2

GC ECD: Gas chromatography with electron capture detector.
HPLC DAD: High-performance liquid chromatography with photodiode array detection.
HPLC UV: High-performance liquid chromatography with ultraviolet detector.
HPLC Fluor: High-performance liquid chromatography with fluorescence detector.
High-performance liquid chromatography with electrochemical detector.
HPLC MS/MS: High-performance liquid chromatography with mass spectrometry.
GC MS/MS: Gas chromatography with mass spectrometry.

4.2. REQUIRED LIMITS

The Service Laboratories must consider as Required Limits those values of DL or QL equal to or lower than those reported by the Verification Laboratory of SERNAPESCA (table). These limits will set the minimum requirement for the reading capacity required by the analysis methods of the Service laboratories and do not limit their maximum capacity. Those laboratories with validated DL or QL lower to those informed by the Verification Laboratory of SERNAPESCA must inform according to those limits.

In the case of substances with a defined tolerance, a quantification limit of the analysis method of at least 50% of the MRL is required. Due to the aforementioned, a DL is not required from analysis laboratories for these types of substances. For those substances that do not have a tolerance level, and therefore must be absent from the sample, the laboratory is required to have a minimum capacity to detect the presence of the analyte. For this reason, its quantification depends on the validation of the method applied by the laboratory.

4.3. DETERMINATION OF DETECTION AND QUANTIFICATION LIMITS

The detection and quantification limits for each analysis technique must be defined according to the following criteria:

a) "The limit detection is the smallest measured concentration of an analyte from which it is possible to deduce (...) the presence of the analyte in the test sample. This determination should consider matrix related interferences with an instrumental signal to noise (S/N) ratio greater than 5:1 or the concentration determined by a factor of 3 standard deviations of the signal response for blank tissue, whichever is less." Codex Alimentarius.
b) The limit of quantification of a method will be determined by a factor of 10 standard deviations of the signal response for blank tissue, or the lowest quantifiable signal with a variation coefficient (VC) lower than or equal to 10%.

4.4. INTERPRETATION OF THE RESULTS

The following criteria are set to standardize the residues analysis and results reporting procedures in private service laboratories:

a) When the results are below the detection limit, they will be reported as Not Detected (ND).
b) If the result is between the DL and QL, it will be reported as Traces (T).
c) If the result is above the QL of the technique, the concentration of the analyte must be quantified, and the concentration will be reported in ppb or ng/g.
d) For all purposes, the traces (T) will be considered as positive results, with the presence of the specific residue.
e) In the case of official verifications of the residues control plan, if a positive result is obtained, all the samples corresponding to that Samples Delivery Form will be analyzed to confirm the presence, exclusively, of the originally detected analyte. If again, a positive result is obtained in one of the samples, the result of the verification will be considered as unfavorable. If the analyte is not detected in any of the samples, the result will be considered as suspicious, and SERNAPESCA may request a new sampling to the company. In parallel, SERNAPESCA will require the laboratory to review its records and assess the possible causes of the discrepancy if it came from the laboratory.

4.5. RESULTS QUALITY CONTROL

To conduct a proper quality control of the results, the analysis laboratories must set up a results acceptance procedure, which must comprise at least the following analysis logbook for routine tests and must set the limits for each control stage:

a) 3 injections of analyte standard solution (prepared the same day and corresponding to the solution used to fortify the samples to be analyzed), at the same concentration: verifies the stability of the drug and the operation of the equipment.
b) Mobile phase (verifies impurities in the chromatographic line).
c) Negative control: Blank sample.
d) Positive control (PC): Fortified sample at a DL or QL level.
e) Calibration curve of five fortified samples of different concentrations. They are injected from the lowest to the highest concentration. The one with the lowest concentration must correspond to the detection limit of the technique.
f) Repeat steps a, b and d. In this case, only one injection should be considered for "a."

Items a, b and d must be repeated every 20 analyzed samples. Item d will be used to keep a recovery control for each batch of samples.

A graphic control of the PC areas must be kept.

The laboratory must establish a procedure to repeat at random and blinded for the analysts, 5% of the samples analyzed on a daily basis (considering equal percentages of positive and negative samples).

Each stage of the quality control process must have clearly defined approval and rejection limits.

For the specific case of the SERNAPESCA Verification Laboratories, they must send at least twice a year against samples to an International Reference Laboratory designated by the Service, in order to verify the results obtained. The countersample will be selected by SERNAPESCA.

4.6. QUANTIFICATION OF THE RESULTS

If a positive result is obtained, the detected sample must be quantified according to one of the following protocols:

a) Having satisfactorily complied with the quality control of the results, as stated in item 6, the results will be quantified by comparison with a fortified sample calibration curve of at least 5 points, which must be conducted on the same day in which the sample analysis takes place. A point can be eliminated from this curve for a better adjustment, but the points at the ends may never be eliminated from the curve. It must be controlled that the conditions of the fortified curve are maintained over time through control letters of the curve's slope and of the area of the fortified sample within the range of work.
b) Having satisfactorily complied with the quality control of the results, as stated in item 6, the results will be quantified by comparison with a standards calibration curve of at least 5 points in the range of work concentrations and correction by the recovery factor. A daily control of the method's recovery must be kept.

4.7. MINIMUM REQUIREMENTS FOR PROCESSING SAMPLES

a) The laboratory is responsible for verifying that the sample received complies with the conditions set forth in Chapter II, Item 2.1.3.5.
b) Afterward, and to obtain the sample to be analyzed in the laboratory, the received samples will be chopped, moving from point A to point B, as necessary, to obtain the quantity of flesh required for the analysis, as shown in figure 2.
c) The counter sample will be obtained from the opposite half of the sample for analysis, as shown in figure 2. Before its storage, the osseous and cartilaginous tissue of the counter sample must be removed.
d) Grinding and homogenization of the samples for residues analysis must be conducted with a food grinder or a *blender*.
e) If the matrix to be analyzed is muscle and skin in natural proportions, only the skin of the piece to be homogenized must be analyzed. The skin should not be separated from the muscle, to weigh it and process it separately. Cuts can be made in the skin with a scalpel to facilitate its homogenization.
f) Stainless steel knives with steel handles will be used.
g) All tools must be washed with detergent in between samples, even if they come from the same cage.

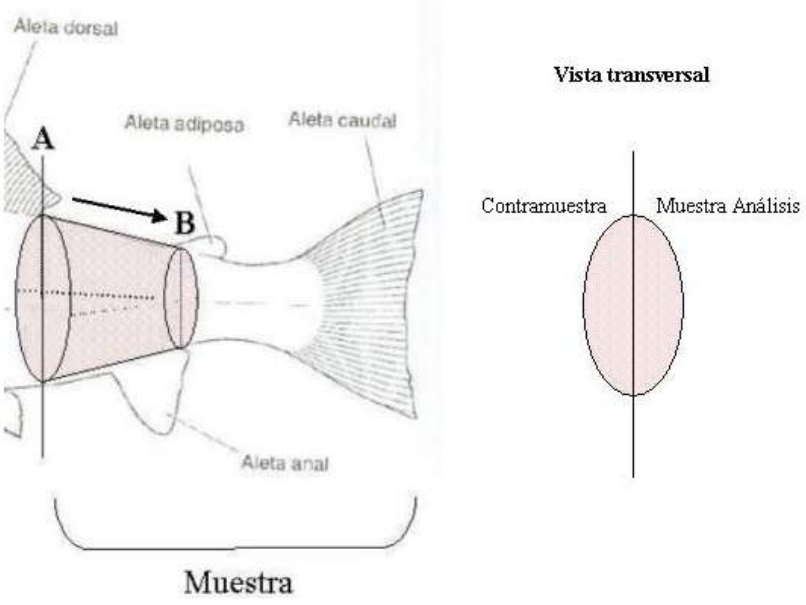

Figure 2: Schematics for obtaining the sample and counter samples of fish for the analyses of pre-harvesting residues.

4.8. REGARDING THE COLLECTION PROCEDURES

a) The use of ultrasound is recommended for the dissolution of the sample. Sonicators can substantially improve the recovery of the analyte and the technique's detection capability.
b) It is recommended to dry the samples by evaporation of gaseous nitrogen in the analyte concentration process.
c) In the case of solid phase extractions, the extraction cartridges cannot be reused.
d) It is recommended to use disposable containers if these are going to come into contact with the samples.

4.9. BIBLIOGRAPHY REFERENCES OF THE METHODS

a) Quinolones (Oxolinic acid, Flumequine, Danofloxacin, Ciprofloxacin, Enrofloxacin, Pefloxacin, Ofloxacin, and Norfloxacin):

- Rapid assay monitoring residues of enrofloxacin and sarafloxacin in fish tissues by High-Performance Liquid Chromatography. Victor Hormazabal, Artri Logdad, Ingrid Steffenak, and Magne Yndestad. Journal of Liquid Chromatography 14 (8), 1605-1614, 1991.
- Simultaneous determination of Quinolones in livestock and marine products by high-performance liquid chromatography. J. Food Drug Analysis, 11 (2): 114-127, 2003.
- Simultaneous determination of quinolones in fish by liquid chromatography coupled with fluorescence detection: comparison of sub-2μm particles and conventional C18 columns. J. Sep. Sci., 33: 1959-1967. 2010

b) Tetracyclines (Tetracycline, Oxytetracycline, Chlortetracycline, 4-epi-Tetracycline, 4-epi-Oxitetracycline, 4-epi-Clortetracycline, Doxycycline):

- Analysis of tetracyclines in honey by high-performance liquid chromatography/tandem mass spectrometry. Seu-Ping Khong, et al. Rapid Common. Mass Spectrom. 2005; 19: 493-502.
- Detection of tetracycline and oxytetracycline residues in pig and calf hair by ultra-high-performance liquid chromatography-tandem mass spectrometry. J. Chrom. A., 1216:8096-8100. 2009.

- Determination of tetracyclines in tissue by HPLC-DIODE-ARRAY. Instituto de San Carlos III. Centro Nacional de Alimentación. Dirección General de Salud Alimentaria y protección de los Consumidores (General Directorate of Food Health and Consumers Protection). Food residues analysis methods. Method 3.9.
- Method for determination of OTC residues in uncooked shrimp using HPLC. FDA December 1999. 15.
- A confirmatory method for the determination of tetracyclines in muscle using high-performance liquid chromatography with diode-array detection. 2009. Analytica Chimica Acta. 637, 40-46.

c) Florfenicol and Florfenicol Amine:

- Simultaneous determination of residues of florfenicol and florfenicol amine in fish tissues by High-Performance Liquid Chromatography. Hormazabal, V.; Steffenak, I.; Yndestad, M. Journal of Chromatography. 616: 161 165. 1993.

d) Sulfonamides:

- Multi-residue methods for the determination of sulphonamides in pork tissues. J. Agric. Food Chem. Vol 38, Nº 2. Austin R. Long; Lily C. Hsieh; Malbrough; Charles R. Short and Steven A. Barke. J. Agric. Food Chem. Vol 38, Nº 2. 1990.

e) Erythromycin:

- Bibliographical reference: P. Edder, L. Coppex, A. Cominoli, C. Corvi. Analysis of erythromycin and oleandomycin residues in food by high-performance liquid chromatography with fluorometric detection.

f) Steroids and Resorcylic Acids:

- The Merck Index 12″ Edition, 1996; Veterinary Drug Residues. Edited by Rod Heitzman, 2nd Edition, 1994; Methods Standard Operating Procedures. Centre for Veterinary Drug Residues. March 14, 1997; Royball et al., JAOAC. Vol 71, Nº2, 1998, page 263 –271; Daeseletre E. Journal of Chromatography. 562, 1991, pages 673 679.

g) Chloramphenicol:

- Analytic procedure for the determination of Chloramphenicol by gas chromatography. 1991. United States Department of Agriculture Analytical Chemistry Laboratory Guidebook. Residue Chemistry, Second Edition. 1998.

h) Nitrofurans:

- Matrix solid-phase dispersion (MSPD) isolation and liquid chromatographic determination of furazolidone in pork muscle tissue. Long, A.R.; Hsieh, L. C.; Malbrough, M.S.; Short, C.R.; Barker, S.A. J. Assoc. Off. Anal. Chem. 74 (2): 292 294. 1991.

i) Dimetridazole:

- Determination of dimetridazole, romidazole and their common metabolite in poultry muscle and eggs by High-Performance Liquid Chromatography with UV detection and confirmatory analysis by atmospheric pressure chemical ionization mass spectrometry. Martin J. Sams, Paul R. Stritt, Karen A. Barnes, Andrew P. Domant and Martin D. Rose. 1998. Analyst, 123: 25452549.

j) Organochlorines:

- Matrix solid-phase dispersion extraction and gas chromatographic screening of 14 chlorinated pesticides in oysters (Crassostrea virginica). Lott, H.M.; Barker, S.A. Journal of AOAC International. 76 (1): 67 72. 1993.

k) Diquat:

- Simple method for analysis of Diquat in biological fluids and tissues by High-Performance Liquid Chromatography. Madhu, C.; Gregus, Z.; Klaasen, C.D. Journal of Chromatography B. 674: 193196. 1995.

l) 2,4D:

- Toxicology of 2,4-Dichlorophenoxyacetic acid (2,4-D) and its determination in serum and brain tissue using gas chromatography-electron capture detection. G.H. Oliveira and J. PalermoNeto. Journal of Analytical Toxicology. Vol 19. 1995.
- Simultaneous determination of 2,4 D and MCPA in canine plasma and urine by HPLC with fluorescence detection using 9Anthryldiazomethane (ADAM). Laurence M. Dickow, Diane F. Gerken, Richard A. Sams and Susan M. Ashcraft. Journal of Analytical Toxicology. Vol 25. 2001.

m) Dichlorvos:

- Matrix solid-phase dispersion extraction and gas chromatographic screening of 14 chlorinated pesticides in oysters (Crassostrea virginica). Lott, H.M.; Barker, S.A. Journal of AOAC International. 76 (1): 67 72. 1993.

n) Emamectin Benzoate:

- Quantitation of Emamectin Benzoate Residues in salmon muscle with skin attached in natural proportions. Claire McCambley, Richard Enndris. Schering Plough Animal Health Corporation. New Jersey. 1999.

o) Amoxicillin:

- FSIS Analytical Chemistry Laboratory Guidebook-Residue Chemistry. 1991. Amoxicillin. AMX1-AMX7.

p) Nitroimidazoles:

- SOP FOR THE DETERMINATION OF NITROIMIDAZOLES IN TISSUES, MILK, AND EGGS. R. McCracken. AFBI, Veterinary Sciences Division, Stoney Road, Stormont, BELFAST BT4 3SD.
- Martin J Sams, Paul R. Strutt, Karen A. Barnes, Andrew P. Dammant and Martin D. Rose. 1998. Determination of dimetridazole, ronidazole and their common metabolite in poultry muscle and eggs by high-performance liquid chromatography with UV detection and confirmatory analysis by atmospheric pressure chemical ionization mass spectrometry. Analyst, 123, 2545 - 2549.

q) Steroids:

- Dickson, L.C., MacNeil, J.D., Reid, J., Fesser, A.C.E. 2003. Validation of Screening Method for Residues of Diethylstilbestrol, Dienestrol, Hexestrol and Zeranol in bovine urine using immunoaffinity chromatography and gas chromatography/mass spectrometry. J. AOAC Int 86(4): 631-639.
- Impers, S., De Waseh, K., Cornelis, M., De Brabander, H.F. 2002. Analysis on residues of estrogens, gestagens, and androgens in kidney fat and meat with gas chromatography-tandem mass spectrometry. J. Chrom A 970: 235-247.

r) Malachite Green, Crystal Violet, Brilliant Green and their metabolites (HPLC-DAD):

- Jose E. Roybal, Allen P. Pfenning, Robert K. Munns, David C. Holland, Jeffrey A. Hurlbut and Austin R. Long. Determination of Malachite Green and Metabolite, Leuco Malachite Green In Catfish (Ictalurus punctatus) Tissue by High-Pressure Liquid Chromatography with Visible Detection (LC/VIS). Food and Drug Administration. Animal Drugs Research Center. Denver Federal Center. 2001. 15 pp.

- Jonathan A. Harbin, Karen A. Barnes, John Bygrave and William H. H. Farrington. Screening and confirmation of triphenylmethane dyes and their leuco metabolites in trout muscle using HPLC-vis and ESP-LC-MS. Analyst, 1998, 123, 2567-2571.

s) Malachite Green, Crystal Violet, Brilliant Green and their metabolites (HPLC-MS-MS):
- Bergwerff AA, Scherpenisse P. Determination of residues of malachite green in aquatic animals. J Chromatogr B Analyt Technol Biomed Life Sci. 2003 May 25;788(2):351-9.
- Dowling G, Mulder PP, Duffy C, Regan L, Smyth MR. Confirmatory analysis of malachite green, leucomalachite green, crystal violet and leucocrystal violet in salmon by liquid chromatography-tandem mass spectrometry. Anal Chim Acta. 2007 Mar 14;586(1-2):411-9.
- Jonathan A. Harbin, Karen A. Barnes, John Bygrave and William H. H. Farrington. Screening and confirmation of triphenylmethane dyes and their leuco metabolites in trout muscle using HPLC-vis and ESP-LC-MS. Analyst, 1998, 123, 2567-2571.

t) Cypermethrin and Deltamethrin:
- Audrey W. Chen, J. Michael Fink, David J. Letinski, George P. Barrett, and Jill C. PearsallJ. Analytical Methods to Determine Residue of Cypermethrin and Its Major Acid Metabolites in Milk and Tissues from Dairy Bovines Treated with Cypermethrin. Agric. Food Chem. 1997, 45, 4850-4855

u) Diflubenzuron and Teflubenzuron:
- Rodriguez E., Barrio RJ., Goicolea A., Gomez de Balugera Z. Determination of Diflubenzuron and its main metabolites in forestry matrices by liquid chromatography with on-line diode-array and electrochemical detection. Analytica Chi,ica Acta 384 (1999) 63-70.

v) Aminoglycosides (Neomycin and Spectinomycin):
- Simultaneous determination of 13 aminoglycoside residues in food of animal origin by liquid chromatography-electrospray ionization tandem mass spectrometry with two consecutive solid-phase extraction steps. Journal of Chromatography A, 1207 (2008) 29-37.

5. PHYTOPLANKTON ANALYSIS METHODS

The following section includes the official methods recognized by SERNAPESCA, for conducting the phytoplankton analysis, for classifying and monitoring bivalve mollusks extraction areas, according to the Bivalve Mollusks Sanitation Program.

5.1. PHYTOPLANKTON ANALYSIS METHODS

5.1.1. QUALITATIVE ANALYSIS PROCEDURE

The analysis must be conducted on a sediment sample for a minimum of 3 hours. Three replicate samples of the decanted net of 0.1 ml each will be analyzed, using an 18 x 18 mm coverslip. The result of the analysis corresponds to the average of the number of cells of the replicate samples.

The microscopic observation and analysis must be done with a phase-contrast microscope. The use of other types of microscopes will be allowed to the extent that they provide a similar or better image than the phase-contrast microscope, and if its technology is validated for analysis by recognized scientific journals.

The result will be marked at the corresponding level of the relative abundance table.

Due to the differences between the taxa and their interrelationships, the scale cannot be unique. The scale applied to the main harmful species is the following:

Table : *Relative Abundance Scales*

	Scale	*D. acuta, D. acuminata, Alexandrium ostenfeldii, Protoperidinium crassipes, Prorocentrum micans.*	*A. catenella, Protoceratium reticulatum*	*Pseudo-nitzschia spp., australis and pseudodelicatissima*
Absent	0	0	0	0
Rare	1	1 – 5	1 – 2	1 – 10
Scarce	2	6 – 15	3 – 10	11 – 50
Regular	3	16 – 35	11 – 42	51 – 210
Abundant	4	36 – 75	43 – 170	211 – 850
Very Abundant	5	76 – 155	171 – 682	851 – 3,410
Extremely Abundant	6	156 – 315	683 – 2,730	3,411 – 13,650
Hyper Abundant	7	316 - 635	2,731 - 10,922	13,651 – 54,610

5.1.2. Quantitative Analysis Procedure

The Utermöhl method (1958) must be applied for the determination of cells using sedimentation buckets.

The minimum volume used will be of 10 ml, and will increase (20 ml), depending on the abundance of the sample.

The decantation time should be estimated for the different volumes. In the case of a 10 ml sample, this must be decanted for a minimum of 6 hours.

The cell count will be done with an inverted microscope and preferably with phase contrast. The objective to be used must be of 10x or 20x if the entire field is counted.

The results must be expressed in cells per liter (cell/L).

6. MICROBIOLOGICAL ANALYSIS METHODS FOR EXPORT FISHERY PRODUCTS

The following section describes the official technical standards recognized by SERNAPESCA, based on which laboratories must implement the microbiological analysis methods for export fishery products.

6.1. AEROBIC MESOPHILES

a) Application:

This method applies to fishery products in general.

b) Method:

- NCh 2659.Of2002: Hydrobiological products – Determination of mesophilic aerobic microorganisms - Plate-count technique at 35 C°.

c) Reference:
- Bacteriological Analytical Manual, 1995, Food and Drug Administration Chapter 3: Aerobic Plate Count.

6.2. *ESCHERICHIA COL*I β-GLUCURONIDASE-POSITIVE

a) Application:
This method applies to fishery products in general and mollusks, gastropods, echinoderms and live tunicates controlled within the context of the Bivalve Mollusks Sanitation Program.
A joint sample of a minimum of 10 animals and five tubes per dilution must be considered for live bivalve mollusks.
The expression of the results must be based on the tables included in the applicable ISO 7218 Standard.
The Tryptone Bile Glucuronide agar plates can only be stored for up to five days at the temperature described in the standard. For SERNAPESCA inspection purposes, the laboratory must have a preservation record system for this medium.
b) Method:
- NCh 3056 Of.2007. Horizontal method for the enumeration of β -glucuronidase-positive Escherichia coli. Most Probable Number technique using 5-bromo-4-chloro-3-indolyle β -D-glucuronide.

c) Reference:
- Chapters 1 and 2, Annex 1, Regulation (EC) 2073/2005 of the European Union.
- ISO/TS 16649-3 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β – glucuronidase – positive *Escherichia coli* –
- ISO 6887-3: Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products.
- ISO 7218: Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations.

6.3. TOTAL COLIFORMS

a) Application:
This method applies to fishery products in general. In the specific case of fish oil, special care must be put in the initial dissolution stage of the sample, using the regulations in force for these purposes.
b) Method:
- NCh 2635/1.Of2001: Determination of Coliforms – Part 1: Determination of Coliforms and Fecal Coliforms – Most Probable Number (MPN) Techniques.
- ISO 4831:1991: Microbiology-General guidance for the enumeration of coliforms-Most probable number technique.

c) Reference:
- ISO 4831:1991: Microbiology-General guidance for the enumeration of coliforms-Most probable number technique.
- Bacteriological Analytical Manual, 1995, Food and Drug Administration (FDA).
- Manual del Instituto de Salud Pública de Chile, 1998.

6.4. MOLD AND YEAST

a) Application:
This method applies to fishery products in general.

b) Method:
- NCh 2734.Of2002: Hydrobiological products – Determination of mold and yeast - Plate-count technique.

c) Reference:
- ISO 13681: 1995 Meat and meat products – Enumeration of yeasts and molds - Colony- count technique.
- Manual de técnicas Micobiológicas para Alimentos y Aguas, 1998, Mohos y/o levaduras, Instituto de Salud Pública de Chile (Manual of microbiological techniques for food and water, 1998, molds and yeasts, Public Health Institute of Chile).

6.5. *STAPHYLOCOCCUS AUREUS* (DETECTION) EURASIAN ECONOMIC UNION

a) Application:

This method applies to fishery products in general. In the specific case of fish oil, special care must be put in the initial dissolution stage of the sample, using the regulations in force for these purposes.

For the confirmation of *S. aureus*, additionally, the confirmation tests for the detection of acetoin formation and the fermentation of maltose must be applied, as described in items 9.5.1 and 9.5.2 of Russian standard GOST 31746-2012 Methods for Detection and Quantity Determination of Coagulase-Positive Staphylococci and *Staphylococcus Aureus*.

The dissolution to be made, and the way in which the results must be expressed, depends on the presentation of the product (see Section III, Chapter IV, Item 2.35), as detailed in the following table:

Table: *To inform the results of absence in 1.0, 0.1, 0.01 or 0.001 grams of product.*

Take 10^{-1} of dissolution (ml)	Take (ml)	Expression of results
10*	10 of dissolution 10^{-1}*	Absence/1 gr
1.0**	1 of dissolution 10^{-1}**	Absence/0.1 gr
0.1**	1 of dissolution 10^{-2}**	Absence/0.01 gr
0.01**	1 of dissolution 10^{-3}**	Absence/0.001 gr

* Add at 10ml of double strength concentration Giolitti-Cantoni Broth.

* Add at 10ml of simple concentration Giolitti-Cantoni Broth.

b) Method:
- ISO 6888 – 3:2003 Microbiology of food and feedingstuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - part 3: Detection and MPN technique for low numbers.
- GOST 31746-2012 Methods for Detection and Quantity Determination of Coagulase-Positive Staphylococci and *Staphylococcus aureus*.

c) Reference:
- ISO 6888 – 3:2003 Microbiology of food and feedingstuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 3: Detection and MPN technique for low numbers.
- GOST 31746-2012 Methods for Detection and Quantity Determination of Coagulase-Positive Staphylococci and *Staphylococcus aureus*.

6.6. COAGULASE-POSITIVE STAPHYLOCOCCI (*STAPHYLOCOCCUS AUREUS* AND OTHER SPECIES) (ENUMERATION)

a) Application:

This method applies to fishery products in general. In the specific case of fish oil analyses, 1 g of oil must be seeded in agar Baird Parker, without dissolutions, and the results in the case of absence must be informed lower than DL/g, that is to say, lower than 1 ufc/g (item 11.2.2).

b) Method:

- NCh 2671.Of2002: Hydrobiological products – Coagulase-Positive *Staphylococcus aureus* – Plate-count technique in Baird-Parker agar.

c) Reference:

- ISO 6888-1: 1999 Microbiology of food and animal feedingstuffs – Horizontal method for the enumeration of coagulase – positive staphylococci (Staphylococcus aureus and other species) – Part 1: Technique using Baird-Parker agar medium.

6.7. *SALMONELLA SPP*

a) Application:

This method applies to fishery products in general. In the specific case of fish oil, special care must be put in the initial dissolution stage of the sample, using the regulations in force for these purposes.

b) Method:

- NCh 2675.Of2002: Hydrobiological Products - Detection of Salmonella.

c) Reference:

- ISO 6579: 1993 (E), Microbiology – General Guidance for the detection of Salmonella.
- Bacteriological Analytical Manual, FDA, 2001
- Manual of microbiological techniques for food and water, Public Health Institute of Chile.

6.8. *LISTERIA MONOCYTOGENES* (DETECTION)

a) Application:

This method applies to fishery products in general.

b) Method:

- NCh 2657.Of2001: Hydrobiological products – detection of *Listeria monocytogenes*.

c) Reference:

- ISO 11290-1: 1996, Microbiology of food and animal feedingstuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method.
- FDA Bacteriological Analytical Manual 1992, Chapter 10 *Listeria monocytogenes*.

6.9. *LISTERIA MONOCYTOGENES* (ENUMERATION)

a) Application:

This method applies to fishery products in general.

b) Method:

- NCh 2657/2.Of2007- Microbiology of food and animal feedingstuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration methods.
- ISO 11290-2: 1998/Amd 1: 2004, Microbiology of food and animal feedingstuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method.

c) Reference:

- ISO 11290-2: 1998(E), Microbiology of food and animal feedingstuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*.
- Part 2: Enumeration method. Amendment 1 2004-10- 15

6.10. *BACILLUS CEREUS*

a) Application:
This method applies to fishery products in general.
b) Method:
- FDA Bacteriological Analytical Manual, online 2001.

c) Reference:
- FDA Bacteriological Analytical Manual, online 2001.

6.11. *CLOSTRIDIUM PERFRINGENS*

a) Application:
This method applies to fishery products in general.
b) Method:
- FDA Bacteriological Analytical Manual, online 2001.

c) Reference:
- FDA Bacteriological Analytical Manual, online 2001.

6.12. *VIBRIO PARAHAEMOLYTICUS*

a) Application:
This method applies to fishery products in general.
b) Method:
- Enumeration of *Vibrio parahaemoliticus* by the Most Probable Number (MPN) method.

c) Reference:
- FDA Bacteriological Analytical Manual, on line 2004

6.13. ENTEROBACTERIACEAE

a) Application:
Methodology applicable to fish meal.
b) Method:
- NCh 2676.Of2002: Hydrobiological products – Determination of Enterobacteriaceae without resuscitation – MPN technique and plate-count technique.

c) Reference:
- ISO 7402:1993 (E) Microbiology – General guidance for the enumeration of Enterobacteriaceae without resuscitation – MPN technique and colony-count technique.
- FDA Bacteriological Analytical Manual 2001.
- Compendium of Methods for the Microbiological Examination of Food, 3rd Ed., American Public Health Association (APHA).

6.14. MESOPHILES AND THERMOPHILES (AEROBES AND ANAEROBES)

a) Application:
This method applies to canned fishery products.
b) Method:
- NCh 2731.Of2002: Hydrobiological products – Canned products – Determination of Aerobic and Anaerobic Mesophiles and Thermophiles.

c) Reference:
- Bacteriological Analytical Manual, Online The Food and Drug Administration. Chapter 21 - An Examination of Canned Foods, 1995.

- Manual de Técnicas Microbiológicas para Alimentos y Aguas, 1998, Capítulo XI. (Manual of Microbiological Techniques for Food and Water, 1998, Chapter XI). Control de Esterilidad Comercial, Instituto de Salud Pública de Chile (Commercial Sterility Control, Public Health Institute of Chile).

6.15. ASPERGILLUS MOLD

a) Application:
This method applies to fish meal.
b) Method:
- NCh 2735.Of2002: Fish meal – Determination of Aspergillus Mold.

c) Reference:
- Manual de Identificación para microhongos comunes en alimentos (Identification manual for common micro molds in food). Eduardo Piontelli L. and María Alicia Toro S.M. Edited by Universidad de Valparaíso, 1994.

6.16. NORWALK VIRUS

a) Application:
This method applies to bivalve mollusks.
b) Method:
- Reverse transcription polymerase chain reaction (RT – PCR)

c) Reference:
- Tsutomu Kageyama et al. "Broadly Reactive and Highly Sensitive Assay for Norwalk-Like Viruses Based on Real-Time Quantitative Reverse Transcription-PCR." Journal of Clinical Microbiology, April 2003, p. 1548-1557.
- ISO/TS 15216-2:2013 Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 2: Method for qualitative detection.

6.17. *SHIGELLA SPP*

a) Application:
This method applies to fishery products in general.
The biochemical confirmation stage may be conducted until item 9.4.3.10 of the standard.
The serological confirmation stage may be conducted by the Public Health Institute (ISP), by sending isolated strains.
b) Method:
- ISO 21567: Microbiology of food and animal feedingstuffs — Horizontal method for the detection of *Shigella spp*.

c) Reference:
- ISO 21567: Microbiology of food and animal feedingstuffs — Horizontal method for the detection of *Shigella spp*.

6.18. TOTAL COLIFORMS - EURASIAN ECONOMIC UNION

a) Application:
This method applies to fishery products in general.
The requirement of SERNAPESCA is only for the detection of total coliforms (item 4.1 and 9.1 of the standard), not for the plate count or the most probable number.

Out of all the initial dissolutions considered in item 2.6.5 of the GOST 26669-85 standard, only the 1:9 dissolution must be used.
To inform the results of absence/presence in 1.0, 0.1, 0.01 or 0.001 grams of product, which depends on its presentation (see Section III, Chapter I, Item 2), the inoculation and incubation, according to item 9.1.2.1 of the standard, must take place considering the specifications described in the following table or any other alternative that allows to inform the results as mentioned previously:

Table: *To inform the results of absence in 1.0, 0.1, 0.01 or 0.001 grams of product.*

Take (10-1) (ml) of initial dissolution.	Take 10 ml of dissolution:	Expression of results
10.0	10-1	Absence/1 gr
1.0	10-2	Absence/0.1 gr
0.1	10-3	Absence/0.01 gr
0.01	10-4	Absence/0,001 gr

b) Method:
- GOST 52816-2007 Food products. Methods for detection and quantity determination of coliforms.

c) Reference:
- ISO 4831 Microbiology of food and animal feedingstuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique.
- GOST 26669-85 Foodstuffs and food additives - Preparation of samples for microbiological analyses.

6.19. MESOPHILIC AEROBIC AND FACULTATIVE ANAEROBIC COUNTS - EURASIAN ECONOMIC UNION

a) Application:
This method applies to fishery products in general.
The following must be taken into account to implement this method:
The requirement of SERNAPESCA is only for conducting the Plate Count (ufc/g), and not for the Most Probable Number.
Of all the culture media cited in item 5.2 of the standard, the laboratory may use the easiest one to purchase in the market.
Out of all the initial dissolutions considered in item 2.6.5 of the GOST 26669-85 standard, only the 1:9 dissolution must be used.
Results must be expressed as described in items 3.1.1 and 5.4 of the GOST 26670-91 Russian standard.

b) Method:
- GOST 10444.15-94 Food products. Methods for the determination of the number of mesophilic aerobes and facultative anaerobes.

c) Reference:
- ISO 4833:2003 Microbiology of food and animal feedingstuffs – Horizontal method for the count of microorganisms – Colony count technique at 30 C°.
- GOST 26669-85 Foodstuffs and food additives - Preparation of samples for microbiological analyses.
- GOST 26670-91 Food products. Methods for cultivation of microorganisms.

6.20. MICROBIOLOGICAL METHODS FOR WATER ANALYSIS

a) Drinking water analyses required by the European Community.
- Total coliforms:

ISO 9308-1:2000. Water quality -- Detection and enumeration of *Escherichia coli* and coliform bacteria -- Part 1: Membrane filtration method.

- *Clostridium perfringens* (including spores):
  Council Directive 98/83/EC of 3 November 1998 Section XI: ICR Microbiology Laboratory Manual.
- *E. coli:*
  ISO 9308-1:2000 Water quality -- Detection and enumeration of *Escherichia coli* and coliform bacteria -- Part 1: Membrane filtration method.
- Enterococci:
  ISO 7899-2:2000. Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method.
- Total coliforms and *E.coli*:
  NCh 2043.Of.98, "Method of simultaneous determination of bacteria Total coliforms and *Escherichia coli* through the chromogenic substrate technique.

b) Raw water analysis required by bivalve mollusks program.

- *E. coli*:
  Microbiological method applicable in waters for the study of contaminated sources detected in the coastline.

6.21. MICROBIOLOGICAL ANALYSIS METHODS FOR THE AIR AND WALLS OF COLD STORES

a) Application:

This method is applicable for the air and walls of cold rooms in the context of the Sanitation Operational Procedures (SOP), as stated in Section II of the Food Safety and Certification Manual. To implement this method, it must be taken into account that the requirement of SERNAPESCA is to conduct the total mold count, and does not include the identification of *Cladosporium, Thamnidium*, or other molds.

b) Method:

- Annex 7: Instructions for the definition and assessment of mold in the air and in the walls of cold stores from the Sanitary Standards for Cold Stores (approved by the State General Sanitary Doctor of the USSR on 09.29.1988, N4695-88) of the Eurasian Economic Union.

c) Reference:

- Sanitary standards for cold stores (approved by the State General Sanitary Doctor of the USSR on 09.29.1988, N4695-88) of the Eurasian Economic Union.

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