STANDARD OPERATING PROCEDURES FOR CYANIDE TESTING USED BY THE PHILIPPINES CYANIDE DETECTION TEST (CDT) LABORATORY NETWORK

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PREFACE

This manual was prepared for the purpose of standardizing the cyanide analysis and detection procedures of the Cyanide Detection Test (CDT) laboratories sponsored by the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) and administered by the International Marinelife Alliance (IMA). All personnel associated with the CDT laboratories should know the importance of these guidelines for conducting laboratory analyses to detect cyanide.

The manual is written in as concise a manner as possible. It should assist not only the chemists, but also the field samplers, fish examiners and laboratory technicians. The methods described in the manual explain the recommended methods for the laboratory procedures that need to be precisely followed, in a step-by-step fashion, to obtain accurate analyses of cyanide ion concentrations in fish tissues and other samples.

INTRODUCTION

Cyanide is a substance that often occurs in combination with other chemicals in the environment. It combines with other substances to form metallo-cyanide, organo-cyanide, or cyano-nitrile complexes (Duodoroff 1976, 1980; Leduc et al. 1982; Leduc 1984, Eisler 1991). Organically-combined cyanides (for example, acetonitrile) are used as solvents and in organic synthesis, but the cyanide group is tightly bound, and there is no free cyanide. Free cyanide can occur as molecular hydrogen cyanide (HCN) or cyanide ion (CN^-).

Cyanide salts such as sodium cyanide (NaCN) and potassium cyanide (KCN) are colorless crystalline solids that have a slight odor of bitter almonds in damp air. These salts readily dissolve in water and are converted to the uncharged hydrogen cyanide (HCN) molecule in most natural waters. This is a function of pH; in solutions more acidic than pH 8.5 (seawater is about pH 8.0), the cyanide ion combines with hydrogen to form HCN (Duodoroff 1976, Leduc 1984). There is evidence in the scientific literature that it is the HCN molecule, which is toxic to aquatic species of fish and invertebrates due to its ability to penetrate cell walls (Duodoroff 1976, 1980).

The widespread and uncontrolled use of sodium cyanide in the Philippines associated with destructive fishing prompted the Philippine Department of Agriculture Bureau of Fisheries and Aquatic Resources (BFAR) to take action. BFAR contracted the IMA to establish cyanide detection test (CDT) laboratories that assist BFAR in monitoring and regulating the trades in live marine-aquarium and food fish. Certified chemists, laboratory technicians, fish biologists, and fishery-officers staff the six CDT laboratories nationwide. The timely reporting of CDT results by IMA to the Philippine government led to the expansion of funding for the CDT laboratories from BFAR and the Fisheries Resource Management Program sponsored by the Asian Development Bank.

The first CDT laboratory was established at BFAR headquarters in Quezon City, Metro Manila, during 1992. A second BFAR/IMA CDT laboratory was established in Puerto Princesa, on the Island of Palawan in 1993. The original laboratory was moved to a new site near the Manila airport in 1994. A third laboratory was established in Zamboanga City (Island of Mindano) during 1995, and a fourth facility established in Palo (Island of Leyte) during 1996. Two CDT laboratories were established respectively in Cebu City (Island of Cebu) and Davao City (Island of Mindanao) during 1997. The IMA maintains and operates the six CDT laboratories (Fig. 1) and four Monitoring, Inspection, and Sampling (MIS) stations under contract to BFAR.

Figure 1. Front of buildings that contain CDT laboratories situated in Manila, Puerto Princesa, Cebu City, Zamboanga City, and Palo-Leyte.

The BFAR/IMA CDT laboratories monitor fishery products (fish, crustaceans, and shellfish) to determine whether they are tainted with cyanide, inspect fish for signs of blast fishing, and compile data concerning the numbers and weight of these marine products being traded within the Philippines.

The CDT laboratories have adopted an internationally recognized procedure for the detection of cyanide. After evaluations during a 20 year period, the test method was described in the book *Standard Methods For the Examination of Water and Wastewater* (section D2036-91) published about every four years by the American Public Health Association (APHA) in conjunction with the American Water Works Association , and the Water Pollution Control Federation (APHA

1992,1998), and published annually with minor revisions and updates by the American Society of Testing and Materials (ASTM) in the *Annual Book of Standards*, Vol. 11.02 (ASTM 1997).

The distillation method, followed by analytical techniques to establish the quantitative cyanide content of the sample has been demonstrated to produce reliable test results. The distillation procedure can be traced to methods used in England as early as 1912 (Williams 1948). It is with slight modifications the Serfass Reflux Procedure first published in 1952. Extensive investigation and testing conducted in Germany has aided in the evaluation of the recommended procedures (Lancy 1967; Leschber 1967, 1968; Leschber and Schlichting 1969; Bucksteeg and Dietz 1969a, b; Weiner and Leiss 1971). Much of the work on the various catalysts was done in the USA under the direction of C.E. Gonter in the Pittsburg Coke and Chemical Laboratory and at the Cyrus William Rice Division of Nuclear Utilities Services (NUS).

Use of the cyanide Ion Selective Electrode (ISE) for the quantitative deter - mination of cyanide in the distillates was investigated during the 1970 period (Frant et al. 1972, Orion Research Inc. 1975, Sekerka and Lechner 1976). Papers were presented before the Division of Environmental Chemistry of the American Chemical Society. Laboratories participated in round robins (inter-laboratory testing) for the Steel Industry Action Committee of the Ohio River Sanitation Commission (Anonymous 1954) and the ASTM during that time period. Statistics from these studies indicate that the cyanide test is linear over the range of 0.03 to 10 mg/L using the Orion cyanide ISE (Frant et al. 1972; ASTM 1987, 1997).

The present operating manual provides detailed instructions on how to determine cyanide concentrations in fish tissues. The method involves digestion of the fish or invertebrate tissue in acid, reflux distillation to separate the cyanide from interfering substances, and the capture of the cyanide ion in a second flask acting as a scrubber containing sodium hydroxide. The CN^- concentration is then measured using a Thermo-Orion cyanide ISE (Model No. 9406BN).

Part 9 - Operational Safety

Cyanide compounds (e.g. NaCN, KCN, and HCN) are highly toxic. Inhalation of cyanide vapor can cause death in a matter of a few minutes. Cyanide is also rapidly absorbed across the skin. The utmost care should be observed when handling toxic chemicals such as strong acids, strong bases, and cyanide-related compounds. Laboratory procedures and protocols must be stringently followed (Appendix A).

9.1 Clothing Protective clothing such as a laboratory coat, mask, and latex gloves must be worn when handling NaCN or KCN tablets and cyanide solutions (Fig 2).

9.2 Toxic Reagents A fume hood must be used to prepare all cyanide solutions and to house the apparatus used for cyanide testing described in this manual.

Part 10 - Glassware for Cyanide Testing and General Considerations

10.1 Volumetric Glassware:

A sufficient number and types of volumetric glassware should be available in the laboratory. A description of the glassware used in the laboratory is given in Appendix B.

Pipettes used are calibrated to deliver (TD) for laboratory work. The pipettes marked with "TD" are only be accurate when the inner surface is scrupulously cleaned so that water wets it immediately to form a uniform film when emptied. Hence, the pipettes are calibrated to deliver the precise volume indicated.

When standard solutions are being prepared, best results can be obtained if the measurement of weights and/or volume of substances are carefully done. The volumetric flasks used should be ones where the volume is specified (e.g., marked 1000 ml); and a line on the neck of the flask shows the level of liquid needed to accurately measure the volume at a given temperature (e.g., 20^{o}C).

10.2 Cleaning Laboratory Glassware:

Laboratory glassware, containers, and other apparatus should be handled with care to minimize breakage during cleaning. Round bottom flasks and other glassware should be scrubbed with a brush while being washed with detergent in warm water, rinsed using tap water and then finally rinsed using distilled water. The glassware should be dried in an oven or inverted and left to dry by dripping on a rack. Each piece of glassware should be stored in a specified place when it is not in use.

Part 11 Preparation of Reagents and Cyanide Standards

All reagents shall conform to the reagent grade specifications of the American Chemical Society (ACS). Other grades may be used provided they are of sufficiently high purity to ensure high accuracy of the determination. Refer to the listing of the chemicals used and the suppliers given in Appendix C. Solid and liquid reagents must be carefully and accurately prepared (Fig. 3) as described below.

11.1 Sodium Hydroxide Solution (for Absorber Tube) Dissolve 40 grams of sodium hydroxide pellets in distilled water (glass distillation not copper distillation apparatus) and dilute to one liter in a volumetric flask. This is equivalent to a one Molar NaOH solution.

11.2 Sodium Hydroxide Diluent Dissolve 1.6 grams of sodium hydroxide pellets in distilled water and dilute to one liter in a volumetric flask. This is equivalent to approximately 0.04 Molar NaOH solution.

Figure 2. Protective clothing worn by laboratory technician working with cyanide.

11.3 Ionic Strength Adjustor (10 M NaOH) Dissolve 40 grams of NaOH pellets in distilled water and bring to volume in a 100 ml volumetric flask using distilled water. Filter the solution through glass wool and transfer it to a polyethylene container.

Caution: Heat is given off during mixing. Prepare an ice water bath to cool the solution.

11.4 Magnesium Chloride Solution Dissolve 510 grams of magnesium chloride hexahydrate in distilled water and dilute to one liter in a volumetric flask.

11.5 Sulfuric Acid Solution (1:1) Slowly add 250 ml of concentrated sulfuric acid to 100 ml of distilled water in a 500 ml using a volumetric flask. Dilute the solution to the 500 ml mark with distilled (or deionized) water.

Caution: Heat is produced during mixing. Hence, the flask should be cooled in a bath of ice water. Progressively dilute the solution with distilled water to allow it to cool to near room temperature (about 20^{o}C). The final 500 ml volume should be the volume read on the flask at room temperature, since heated solutions tend to expand.

Figure 3. Chemist preparing standard solutions from solid reagents in the CDT laboratory.

11.6 Sulfamic Acid Solution Dissolve 133 grams of sulfamic acid in distilled water and dilute to a volume of one liter in a volumetric flask.

11.7 Cyanide Stock Solution (1000 mg/L)

Weigh out exactly 1.8846 grams of reagent grade NaCN crystals Dissolve the crystals in 4 ml ISA and bring the volume to one liter using distilled water. Store this stock solution in a well-sealed flask in a dark cabinet. Use the stock solution to prepare the cyanide standard solutions needed for the calibration. Prepare fresh stock solution every month.

11.8 100 mg/L Cyanide Standard

a) Use a rubber bulb on a pipette or a metered pipetting device under the fume hood to remove 10 ml of the 1000 mg/L stock solution and add 1 ml of ISA (10 Molar NaOH). Then dilute the mixture to 100 ml using distilled or deionized water.

b) Use the 100 mg/L solution to prepare the following standard solutions.

11.9 10 mg/L CN^- Solution-Pipette 10 ml of 100 mg/L CN^- solution, add 1 ml ISA and dilute to 100 ml using distilled water.

11.10 1 mg/L CN^- Solution-Pipette 10 ml of 10 mg/L CN^- solution, then add 1 ml ISA and dilute to 100 ml using distilled water.

11.11 0.1 mg/L CN^- Solution-Pipette 10 ml of 1 mg/L CN^- solution, then add 1 ml ISA and dilute to 100 ml using distilled water.

11.12 0.05 mg/L CN^- Solution-Pipette 50 ml of 0.1 mg/L , then add 1 ml ISA and dilute to 100 ml using distilled water.

Note: It is necessary to carry out these procedures precisely using calibrated pipettes and volumetric flasks.

11.13 Store the cyanide standard solutions in amber-colored sealed jars.

Part 12 Methodology for Detection of Total Cyanide Ion Concentration

12.1 - Scope: This test method covers the determination of cyanide ion in solutions derived from the acid digestion of fish and/or other animal tissue samples. The cyanide test used by the BFAR/IMA laboratories is based on the digestion of animal tissue and the decomposition of most cyanide compounds in the presence of strong acid, magnesium chloride catalyst, and heat during a one hour reflux distillation (ASTM 1996). Hydrocyanic acid (HCN) vapor is released in the digestion flask and passes through the reflux condenser. Cyanide ion (CN^-) is captured in an absorption tube containing sodium hydroxide (NaOH) solution. The high pH (12-13) of the NaOH ensures that the form of cyanide present in the absorption tube is CN^-. The presence of total CN^- in solution can then be determined using various cyanide tests; such as colorimetric, or ion-selective electrode (ISE) methods. The BFAR/IMA laboratories use a cyanide ISE linked to a digital ISE/pH meter to determine cyanide ion concentrations.

12.2 - Cyanide Detection Using Ion-Selective Electrode

The cyanide ISE (Thermo-Orion model #9406BN) consists of a solid membrane with silver compounds bonded into the tip of an epoxy electrode. When the electrode comes in contact with the sample containing ions, an electrical potential develops across the surface of the sensing membrane. The magnitude of the electrical potential is linearly related on a semilog scale to the concentration of CN^-. Hence, higher electronic potentials indicate higher cyanide concentrations in the sample.

To make a measurement, a second reference electrode with a univarying potential is needed to compare with the membrane potential of the sensing electrode (Fig. 3). A solution fills the reference electrode to complete the electric circuit between the sample and the internal cell of the reference electrode. The point of contact between the sample and the filling solution is called the liquid junction. The meter serves to display the readout (in millivolts, pH units or CN^- concentration units) representing the difference of the electrical potentials between the reference electrode and the sensing electrode.

If the background ionic strength is high and constant relative to the cyanide ion concentration, the activity concentration is constant and activity is directly proportional to concentration. Ionic Strength Adjuster (ISA) is added to all cyanide standards and samples so that the background ionic strength is high and constant relative to variable concentrations of cyanide. For cyanide testing the recommended ISA is 10 Molar NaOH.

12.3 - Apparatus

a) Cyanide Distillation Set-up – the reaction vessel is a 1-liter round bottom flask, with an inlet tube and a condenser attached near the top. The inlet tube must be funnel shaped with an 8-mm stem that extends to within 6-mm of the bottom of the flask (Fig. 4). The condenser that is recommended is of the reflux-type such as a Cold-finger condenser or Allihn condenser. The condenser shall be connected to a vacuum line, which has provisions for fine control. The flask should be heated with a heating mantle.

b) The Selective-Ion Meter or combined Selective-Ion/pH meter is equipped to work with a cyanide ISE and reference electrode. The BFAR/IMA laboratories use the Thermo-Orion Model 920A cyanide Selective-Ion/pH meter.

c) Magnetic stirrer with Teflon-coated stirring bars.

d) Volumetric flasks-250 ml capacity.

e) Beakers- 100 ml and 250 ml capacities.

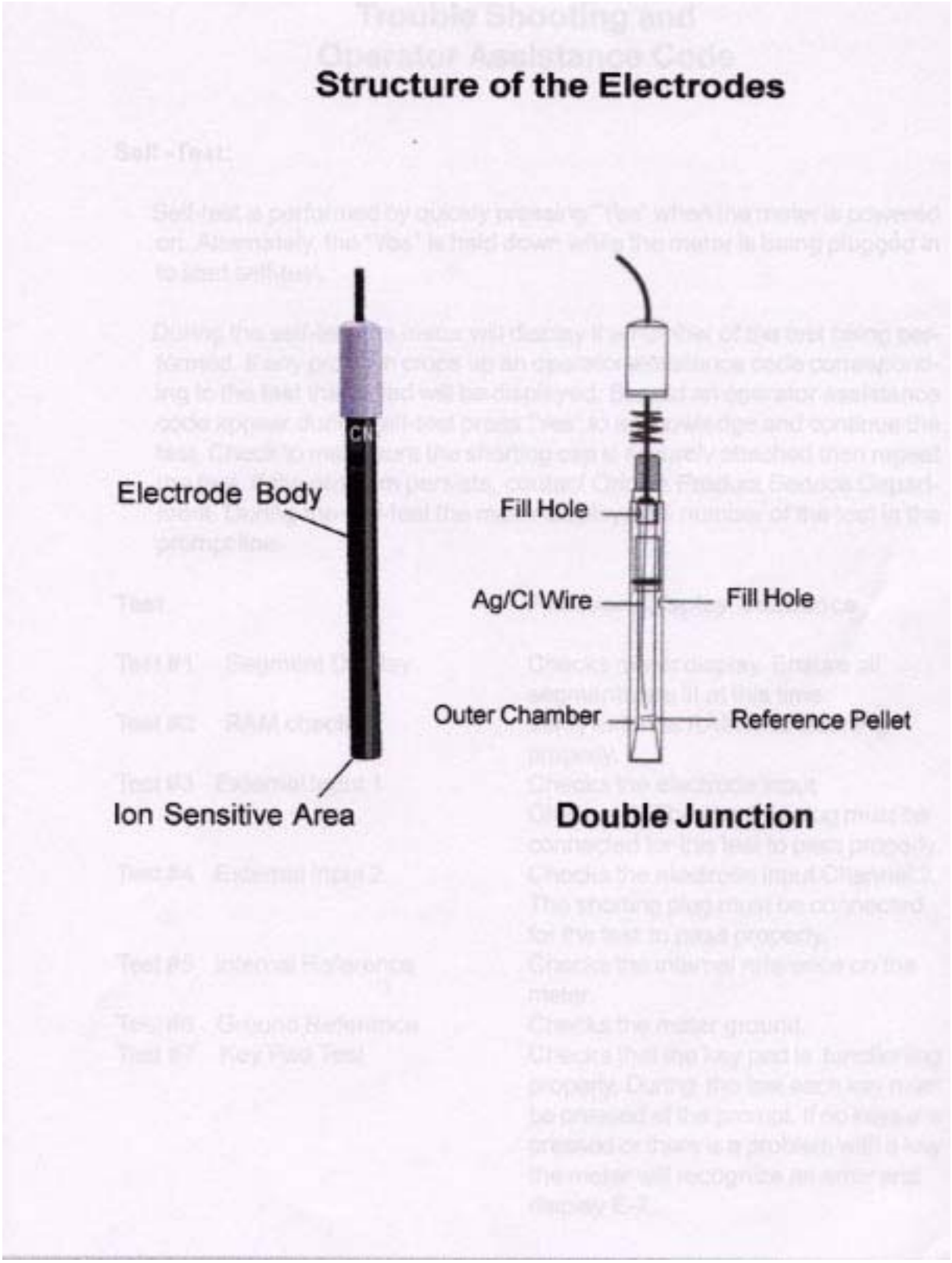


Figure 3. Structure of the cyanide ISE and double junction reference electrodes.

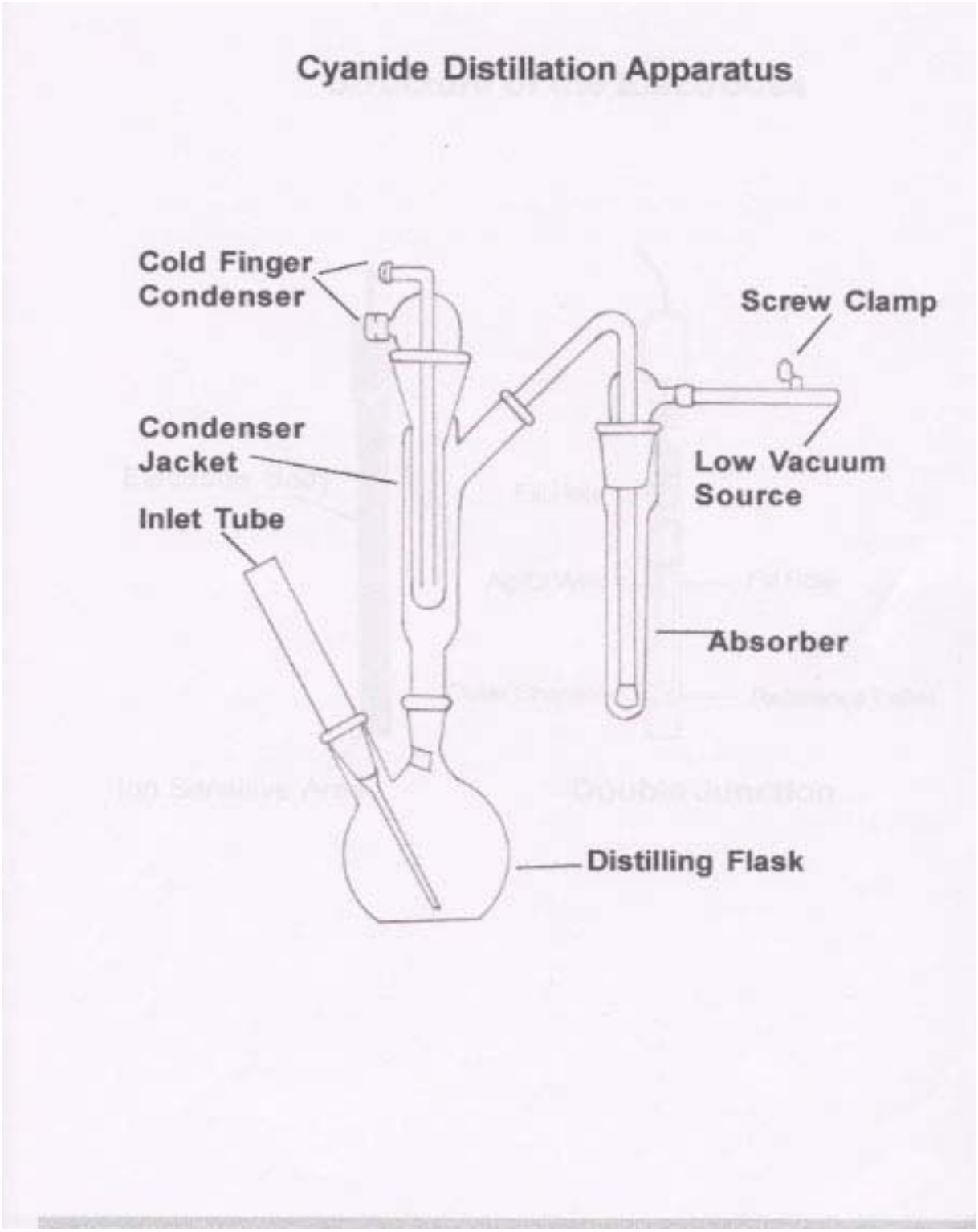


Figure 4. Drawing depicting the cyanide distillation/reflux apparatus used for the total determination of cyanide.

Part 13 - Testing Procedure for Fish Tissues

13.1 Weighing Weigh either the whole fish for small aquarium fish, or the internal organs from groupers for the CDT analysis (Fig. 5). Record the weight of the sample in grams. For fish samples two types of balances are used. Total body weight or large fish is determined using a triple beam balance. Use the top-loading balance for fish tissues intended for distillation. The sample analyzed should weigh approximately 10 grams. See the Sample Preparation SOP for more details.

13.2 Blending Blend the tissue at high speed for 3-5 minutes (Fig. 6) with 2 ml of 10 Normal NaOH diluent and bring it up to 500 ml using distilled water.

13.3 Set up the apparatus as illustrated (Fig. 4).

13.4 Add 10.0 mL of one Molar NaOH solution to the absorber tube. Dilute with distilled water to obtain an adequate depth of liquid. Do not use more than 225 mL total volume of solution in the absorber tube.

13.5 Put about 50 mg (a pinch) of lead carbonate powder into the absorber tube

Note: The lead carbonate is added to precipitate sulfides; which can interfere with the ISE CN^- readings.

Attach the absorber tube to the vacuum and connect it to the condenser (Fig. 4).

13.6 Place the sample (500 ml of blended tissue in solution) into the distillation flask (Fig. 7).

Caution: In the case of unknown substances or solutions, where the cyanide content is suspected to be more than 10 mg/L, use an aliquot of the sample, so that no more than 5 mg of cyanide is added to the distillation flask.

13.7 Connect the flask to the condenser.

13.8 Turn on the vacuum and adjust the airflow to approximately 1-2 bubble per second entering the boiling flask through the air inlet tube. This is approximately 60 ml of air per minute.

13.9 Add 20 ml of magnesium chloride solution through the air inlet tube. About a minute later, add 15 ml of sulfamic acid.

Note: The magnesium chloride acts as a catalyst. The sulfamic acid solution is used to reduce nitrites or nitrates, which can act as interfering substances in the flask.

13.10 Rinse the air inlet tube with a few milliliters of water and allow the air flow to mix the content of the flask for approximately three minutes.

Figure 5. Approximately 10 grams of fish tissue on weighing dish.

Figure 6. Blending approximately 10 grams of whole fish or internal organs of larger fish in sodium hydroxide solution.

Figure 7. Blended fish tissue being added to the distillation flask using a funnel.

13.11 Carefully add 50 mL of sulfuric acid (H_2SO_4) solution (1+1) through the air inlet tube.

Note: Slowly add the acid to the sample. Heat is generated and foaming may occur.

13.12 Turn on the flow of cooling water to the reflux condenser. Heat the solution in the flask to boiling using a heating mantle, taking care to prevent the solution from backing up into the air-inlet tube.

13.13 Maintain the airflow as indicted above. If this airflow does not prevent the sample from backing up into the delivery tube, increase the airflow to two bubbles per second.

Note: Too high a flow of air through the reflux condenser may result in low recovery rates of CN^- in the absorber tube containing NaOH.

13.14 Reflux the sample for one hour (Fig. 8).

13.15 Turn off the heat, but maintain the airflow for at least an additional 15 minutes.

13.16 Transfer the absorption solution from the absorption tube to a 250-mL volumetric flask. Rinse the absorber tube and its connecting tubes sparingly with water and add the rinse water to the volumetric flask.

13.17 Dilute the absorption solution to the 250 mL mark on the flask by adding distilled water and mix thoroughly.

13.18 Determine the concentration of cyanide in the sodium hydroxide solution by using the cyanide ISE linked to the Model 920A PH/ISE meter (Fig. 9) as described in the ISE SOP.

13.19 This method Is applicable in the concentration range of 0.05 to 10 mg/L CN^-.

Figure 8. Reflux apparatus being used to digest fish tissues using sulfuric acid to release HCN vapor, which is then captured in the absorber tube containing sodium hydroxide.

13.20 - Expression of Results

The unit for expressing results of cyanide ion (CN^-) concentrations in fish tissues is in mg/kg. The ISE determines the CN^- concentration per unit volume of solution where one mg/L is equivalent to one part per million (ppm). Hence, adjustments

to the ISE readings need to be made to convert from mg/L to mg/kg. First, divide the weight of the tissue sample in grams by 1000 to express the sample weight in kilograms. Then apply the algorithm given below.

$$\text{Tissue sample cyanide level mg/kg} = \frac{\text{ISE Meter Reading (mg/1000 ml)} \times 250 \text{ ml}}{\text{Weight of Sample Used (kg)}}$$

$$= \frac{\text{ISE Meter Reading (mg)} \times 250/1000}{\text{Weight of Sample (kg)}}$$

Figure 9. Reference and cyanide ISE electrodes connected to the Model 920A pH/ISE meter.

Should the meter reading come out to more than 10 mg/L, make an aliquot of the test solution. Use a 100 ml volumetric flask for the preparation of the aliquot. The diluent required is the 1.6 g/L NaOH solution. Follow the steps previously described for the measurement of samples.

After making each cyanide measurement, remove the electrodes and soak them in distilled water. To prolong the lifetime of the sensing electrode, rinse it well with distilled water, blot it dry, and then replace the rubber cap.

Part 14 – Quality Assurance and Quality Control Procedures

The BFAR/IMA CDT laboratories use laboratory operating protocols developed by the US Environmental Protection Agency (US-EPA) mandated under the Clean Water Act and other US laws to ensure quality assurance and quality control with the cyanide testing (Smith 1995, Berger et al. 1996, Smith 1999a,b).

Quality control is defined as a single step or procedure that is performed to evaluate a single aspect of the analysis or test. Examples of controls are performance evaluations (PE) with blind samples, matrix spikes, and analysis of blanks (Smith 1999a)

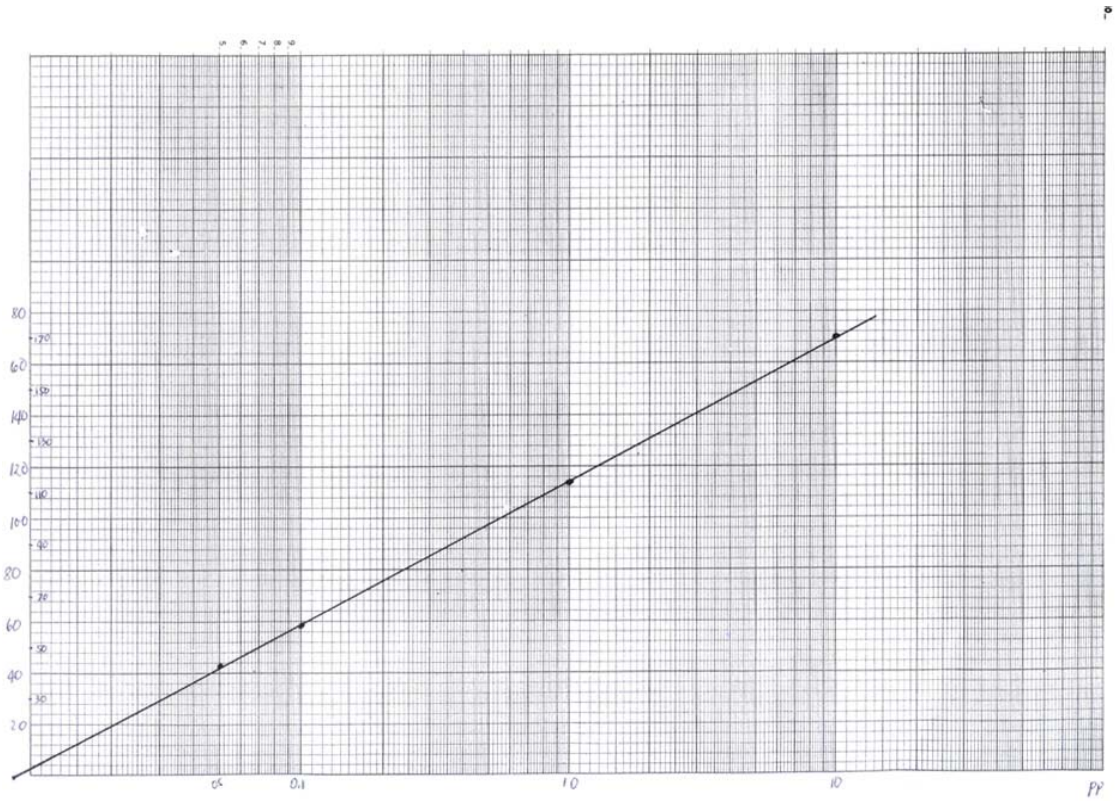
Quality assurance is defined as the sum of all the quality controls performed in the laboratory plus everything else that is done with respect to producing reliable data. The first criterion assesses the ability of the laboratory and the analytical method to perform an analysis within set tolerances (Smith 1999a). This is described as data that are analytically valid. The second criterion assesses the legality of the reported results, i.e., the chain of documents that accompany the sample and verify the actual analysis. This is generally termed the legally defensible aspect of the data.

14.1 Calibration

Calibration is the process where an initial analytical response is related to the amount of analyte present in the sample (Smith 1999a,b). For any calibration there are highest and lowest calibrated points that define the range of the calibration. It is legally non-defensible and poor analytical practice, to report cyanide concentrations of target analytes that are either above or below the range of the calibration unless steps are taken to either concentrate or dilute the solution of the target analyte, and a re-analysis result has been obtained within the calibration range. The reanalysis result is then adjusted for the concentration or dilution factor, and the fact of the concentration or dilution reported on the laboratory worksheet. This requires that if the laboratory is reporting below detection limit (BLD) results for target analyses, then a calibrated point at the concentration of the detection limit must be included on each calibration curve.

Round robin studies between laboratories in the USA determined a calibration range for the cyanide ISE was 0.03 to 10 mg/L (ASTM 1987, APHA 1992). Likewise, the CDT laboratories determined cyanide concentrations were linear on semi-log paper over a four point calibration range of 0.05, 0.10, 1.00, and 10.00 mg/L CN^- (Fig. 10). Other four point calibrations (0.01, 0.10, 1.00, and 10.00 mg/L CN^-) determined that 0.01 mg/L CN^- departed from a straight line relationship.

CALIBRATION CURVE



0.05 0.10 1.00 10.00

CYANIDE ION CONCENTRATION (mg/L)

Figure 11. Graph depicting ISE readings in millivolts on a semi-log scale for various cyanide ion concentrations (0.05, 0.10, 1.00, and 10.00 mg/L). A straight-line calibration was found over the range from 0.05 to 10.0 mg/L CN^-.

Cyanide stock solutions are prepared monthly by each CDT laboratory and stored as previously described. Calibration solutions are prepared weekly. A four-point calibration is conducted in each CDT laboratory on a daily basis.

14.2 Blank Reagents

The CDT laboratory prepare reagent blanks every week, i.e. the distillation is conducted using distilled water + the reagents (except cyanide) to test whether any of the reagents may have cyanide as a contaminant.

14.2 Certified Reference Materials (CRM)

Besides the calibration solutions prepared from the cyanide stock solution by each laboratory, the CDT laboratories also use a certified reference material (cyanide matrix in water obtained from an outside source) to check for the accuracy of the analyses.

14.4 Use of "Blank Fish Samples"

As a baseline or reference level, the CDT laboratory has prepared samples using specimens representative of a variety of marine fish species. The CDT laboratories routinely obtain uncontaminated fish samples from the IMA Net-training teams, who collect fishes with barrier nets. These fish samples routinely test zero indicating that there is no natural background of free cyanide in the marine environment.

14.5 Use of Unknowns

Though intermittently done, the CDT lab conducts parallel testing among the six CDT labs nationwide. This is also our way of counter-checking the test results among the CDT laboratories.

14.6 Use of "Spikes"

The addition of known amounts of cyanide to homogenized fish tissues prior to distillation is regularly undertaken by the CDT laboratories to: a) determine the percent recovery of the distillation process and b) to assess electrode response to cyanide recovered in the distillates. The percent recovery is generally greater than 90%.

14.7 Distillation Efficiency Check

The ASTM method (D2036-98) describes the titration of a 25.0 ml aliquot of the 1000 mg/L NaCN standard solution with silver nitrate (ASTM 1997) This will give the purity of the NaCN and relate it directly to a NIST Primary Standard. Low recoveries of CN^- from distilling aliquots of this standard solution would indicate that the NaCN might have formed hydrates upon storage or HCN is lost during distillation (Williams 1948). If hydrates are formed with NaCN, potassium cyanide (KCN) should be used for the distillation efficiency check.

14.8 Recording of CDT Results

The cyanide concentrations determined are recorded on the data forms (Appendices A and B in the Sample Preparation SOP) and later entered into the CDT database. Test results are classified into four categories. Positive represents CN^- concentrations >0.20 mg/L. Traces are CN^- concentrations from 0.05 to 0.20 mg/L. Cyanide levels >0 and <0.05 mg/L are scored below detectable limits (BDL). Zero values are considered to be Negative. These scores (Positive, Traces, BDL, Negative) should be recorded on CDT Certificates that are returned within 24 hours to those persons who submitted fish for CDT analyses. While most prosecutions deal with fish that tested as being Positive, any level of cyanide found in the fish is an indication that the fish were exposed to cyanide coming from anthropogenic sources.

The names and addresses of suppliers used in the Philippines is given in Appendix D. A list of past and present employees of the BFAR/IMA CDT laboratories and their

professional and academic qualifications is given in Appendix E. A copy of the CDT Certificate issued by the laboratories is given in Appendix F. An overview of the cyanide testing process is presented in Appendix G.

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Appendix A - Rules of Conduct in the Laboratory

1. All laboratory personnel should always wear a clean, white laboratory gown/scrub shirt while working. Laboratory gowns should not be laid onto work tables nor worn at locations where food is eaten.

2. Do not use your bare hands when handling chemicals or samples. Disposable latex gloves are provided for each laboratory to be used by the analytical staff.

a) Preparation of reagents must be done under the fume hood.

b) Do not pour used or unused reagents into the sink or urinal.

3. All used cyanide solutions must be stored in dark-colored bottles with pellets of sodium hydroxide added. The pretreatment of cyanide solutions with NaOH pellets must be done prior to their disposal.

4. Avoid eating, drinking, or smoking while inside the CDT laboratory.

5. Separate combustible substances from non-combustible substances, and organic/halogenated from non-halogenated substances in storage cabinets in the laboratory.

6. Organize the apparatus and other materials before starting work. At the end of each day in the laboratory, clean and return all the apparatus and other materials to specified storage locations.

7. Moisten the table tops with disinfectant and clean the surroundings as previously specified in the SOP manual.

8. Solid wastes such as pieces of plastic, paper, and/or rubber should be placed in baskets or boxes containing disposable plastic bags. The garbage should be removed from the CDT laboratory at the end of each work-day.

9. Loitering and making unnecessary noise should be minimized. The laboratory is a place for work not for recreational activities.

10. All accidents and the breakage of apparatus, such as glassware, should be reported immediately to the laboratory manager. Avoid exposing yourself and others to unnecessary hazards.

11. In case of accidental spills or the splattering of chemicals or samples, promptly rinse the contaminated areas with a large amount of water. Delays in cleaning up the mess may create more serious problems. Immediately, seek medical treatment or advice, when it is needed.

12. Each laboratory should be equipped with a well-stocked first-aid kit, fire

extinguisher, and smoke detector.

Note: Do not work in the laboratory by yourself. There should always be someone else present in case of an emergency.

13. Switch off and unplug all electrical equipment from the electrical outlets prior to leaving the CDT laboratory.

14. Each CDT laboratory should have the following:

    a) First Aid Kit containing antiseptic solutions, antibiotic cream and burn ointments, alcohol, bandages, non-adhesive gauze, dressings, and tape that can be used to treat burns to the skin from heat or cuts from breakage of glassware etc.

    b) Sodium bicarbonate solution-A 50% sodium bicarbonate solution must be available at all times. This is used to wash chemicals such as acids or bases spilled onto the skin. The first aid kit also should contain ointments to treat wounds resulting from chemical spills.

    c) A list the telephone numbers of the nearest hospital/infirmary, physician, police department, fire department, and the numbers for each staff's parents or guardian should be available in each CDT laboratory.

Appendix B Equipment and Glassware Specifications (part A)

Glasswares/equipment	Brand Name/Capacity	Supplier/s
Beaker	"Pyrex"	B.E. Scientific Glass Instrument
	[100, 250, 500, 1000 ml]	
Volumetric Flask	"Pyrex", "Kimax"	B.E. Scientific Glass Instrument
	[10, 50, 100, 250, 500, 1000 ml]	B.E. Scientific Glass Instrument
Volumetric Pipette	"Pyrex", "Fortuna", "Scott Mainz"	B.E. Scientific Glass Instrument
	[1, 2, 5, 10, 20, 25, 50, 100ml]	
Measuring Pipette	"Pyrex", "Kimax","Planax"	B.E. Scientific Glass Instrument
	[10, 5, 1, .1 ml]	
Stirring or glass rod	ordinary- 6mm X 10" long	B.E. Scientific Glass Instrument
Funnel- polyethylene	"Urbanti" Bel-Art Products	B.E. Scientific Glass Instrument
do but glass	"Pyrex"	
Wash Bottle	"Plastibrand" - [500, 250 ml]	B.E. Scientific Glass Instrument
Iron Stand	"Local" - [rust proof]	B.E. Scientific Glass Instrument
Magnetic Stirrer	"Nouva" - Sybron-thermolyne	B.E. Scientific Glass Instrument
Magnetic Stir bars	1" long	B.E. Scientific Glass Instrument
Spatula	"China" porcelain	B.E. Scientific Glass Instrument
		B.E. Scientific Glass Instrument
Trible Beam Balance	"Ohaus" - 2 kg cap.	B.E. Scientific Glass Instrument
Top Load Balance	"Ohaus"- 200 g capacity	B.E. Scientific Glass Instrument
Analytical Balance -	"Setra" EL 200S	B.E. Scientific Glass Instrument
Extension and S- clamp	"Fischer"- Germany	B.E. Scientific Glass Instrument
Brushes e.g. test tube	"Local"	B.E. Scientific Glass Instrument
Rubber Aspirator	"Local"	B.E. Scientific Glass Instrument
Thermometer	"China" - 0-200 °C	B.E. Scientific Glass Instrument
		2

Appendix B Equipment and Glassware Specifications (part B)

Glasswares/equipment	Brand Name/Capacity	Supplier/s
Cyanide Distillation Set-Up	"Pyrex" - 1 liter capacity	B.E. Scientific Glass Instrument
Graduated cylinder	"Pyrex" [10, 20 25,50,100,500 ml]	B.E. Scientific Glass Instrument
Reagents Bottles	"Schott Duran"; "Pyrex" [screw-cap- 250, 500, 1000 ml]	B.E. Scientific Glass Instrument
Dissecting tools	"Stainless Steel/Japan"	B.E. Scientific Glass Instrument
Heating Mantle	"Glascol"; "Whatman" - 1 liter capacity	B.E. Scientific Glass Instrument
Beaker -	Teflon- [100 ml]	B.E. Scientific Glass Instrument
Vacuum Pump	"JB" Fast vac 42l/min - 1/2 HP	B.E. Scientific Glass Instrument
Reagent vials	Screw type- [15, 20 ml]	B.E. Scientific Glass Instrument
AVR	"Autovol" - Jpan Model G1000VA	B.E. Scientific Glass Instrument
pH/ISE Mater	"Orion" - Model 920A [Bench type]	Yana chemodities; Orion [Boston, Mass]
Reference electrode	"Orion" - Model 9002 [Double Junction]	Yana chemodities; Orion [Boston, Mass]
Sensing electrode	"Orion" - Model 9406 BN	Yana chemodities; Orion [Boston, Mass]
others:		
Computer Units	"Intel Inside"- Pentium III	Local Distributors
Fax machine	"Sharp" - Model F0135	
Xerox Copier	"Sharp" - Model ss2314	

Appendix C Reagent Specifications

List of Reagents	Supplier/s	Description/Brand Name/ and its assay
Bleach	Local Grocery Stores	"Zonrox" manuc. by Green Cross Inc, Parañaque, MM
Distilled Water	Local Drug Stores	"Vircon"- Vircon Lab. Inc., Las Piñas, MM
Ethanol	B.E. Scientific Glass Instrument, Theo-Pam	"Riedel de Haen"- 99.8% or its equiv
	Trading Corp., Yana Chemodities,	
Eye Wash	Local Drug Stores	
Formaldehyde	B.E. Scientific Glass Instrument, Theo-Pam	"Univar [Ajax]", [min 36.5; max 38%]
	Trading Corp., Yana Chemodities,	
Inner Filling Solution	Yana Chemodities; Orion [Mass., USA]	"Orion" Cat. No. 900002
Lead Carbonate	B.E. Scientific Glass Instrument & Belman Inc	"J.T. Baker" [101%], "Merck" [99%]
Magnesium Chloride Hexahydrite	B.E. Scientific Glass Instrument & Yana Chem.	"Univar [Ajax]", [min 95; mx 102%] or its equiv.
Outer Filling Solution	Yana Chemodities; Orion [Mass., USA]	"Orion" Cat. No. 900003
Sodium Bicarbonate	B.E. Scientific Glass Instrument	Technical Grade - 50% aq. Sol'n for cleansing spills
Sodium Cyanide	B.E. Scientific Glass Instrument	"J.T. Baker" [99%]
Sodium Hydroxide	B.E. Scientific Glass Instrument & Yana Chem.	"Univar [Ajax]", [97%]
Sulfamic Acid	B.E. Scientific Glass Instrument & Yana Chem.	"Univar [Ajax]" [99.5%]
Sulfuric Acid	B.E. Scientific Glass Instrument & Yana Chem.	"Univar [Ajax]"[min 95%' ; max 98%]
Sodium Hydroxide (flakes)	B.E. Scientific Glass Instrument	Technical Grade - for neutralizing acid waste
Vacuum Pump Oil	B.E. Scientific Glass Instrument	"Robinair" (Ohio, USA)
List of Expendable Supplies	Brand Name	Supplier/s
Latex gloves	"Medic-Dent" or its equiv.	B.E. Scientific Glass Instrument & Local Drug Stores
Particle Masks	Commercial Available- disposable	B.E. Scientific Glass Instrument & Local Drug Stores
Scalpel Blades	"Sovereign", # 20 & # 10	B.E. Scientific Glass Instrument & Local Drug Stores
Fire Extinguisher	ABC-"Kidde Dry Chemical" Model : FA110 G	ABC- "Walter Kidde"
Parafin Film	"Whatman" Cat. # 2150863	B.E. Scientific Glass Instrument
Washing solution	"Rea Phos" [Phosphate free - detergent]	Dispo Philippines
	and "Rea-sol"	

Appendix D Name And Address of Suppliers

Name of Suppliers	Product Lines	Address
Theo-Pam Trading Corporation	Reagents, semi-expendable supplies	2818 P. Celle St., Pasay City
	[Supleco filters, J.T. Baker & Mallinckrodt	
	reagents, ethanol, methanol, acetonitrile,etc.]	
Brownstone Asia- Tech Inc	SPEX "Certi-Prep" - cyanide standard solution	# 10 H. Poblador St., Brgy. Hagdan Bato Libis,
	Cat. No. RSCN9-2X	Mandaluyong City
Yana Cehemodities	Asstd. glasswares, reagents and pH/ISE Meter	#51 Kaliraya St., Quezon city
	[Ethanol, Sulfuric Acid, Magnesium Chloride,	
	Sulfamic Acid, Formalin, Orion pH/ISE meter	
	Filling Solutions, pyrex glasswares]	
B.E. Scientific Glass Instrument	Glasswares, reagents & laboratory equipment	Stall 43 U.P. Shopping Center, U.P. Diliman, Quezon City
	[Heating Mantle(Galsscol), Vacuum Pump (JB),	
	Pyrex Glasswares, Ohaus Balances, J.T. Baker	
	reagents, Merck Reagents, Cyanide Distillation	
	Apparatus]	
J.P. Philippines	Reagents & Vaccum Pump	667 Banawe St., Quezon Ave, Quezon City
	[Lead Carbonate-Merck]; [J.B. Vaccum Pump]	
Dakila Trading Corporation	Instrument and reagents	208 Pilar St., Mandaluyong City
	[HPLC-SFE system, and HPLC accessories	
	(Supelco)]	
Belman Compania Inc	Heating Mantle & Reagents	Cordillera St. cor Quezon Ave, Quezon City
	[Lead Carbonate-Merck]; [Whatmann Heating Mantle]	
		4

Appendix E List of Employees and Qualifications (part A)

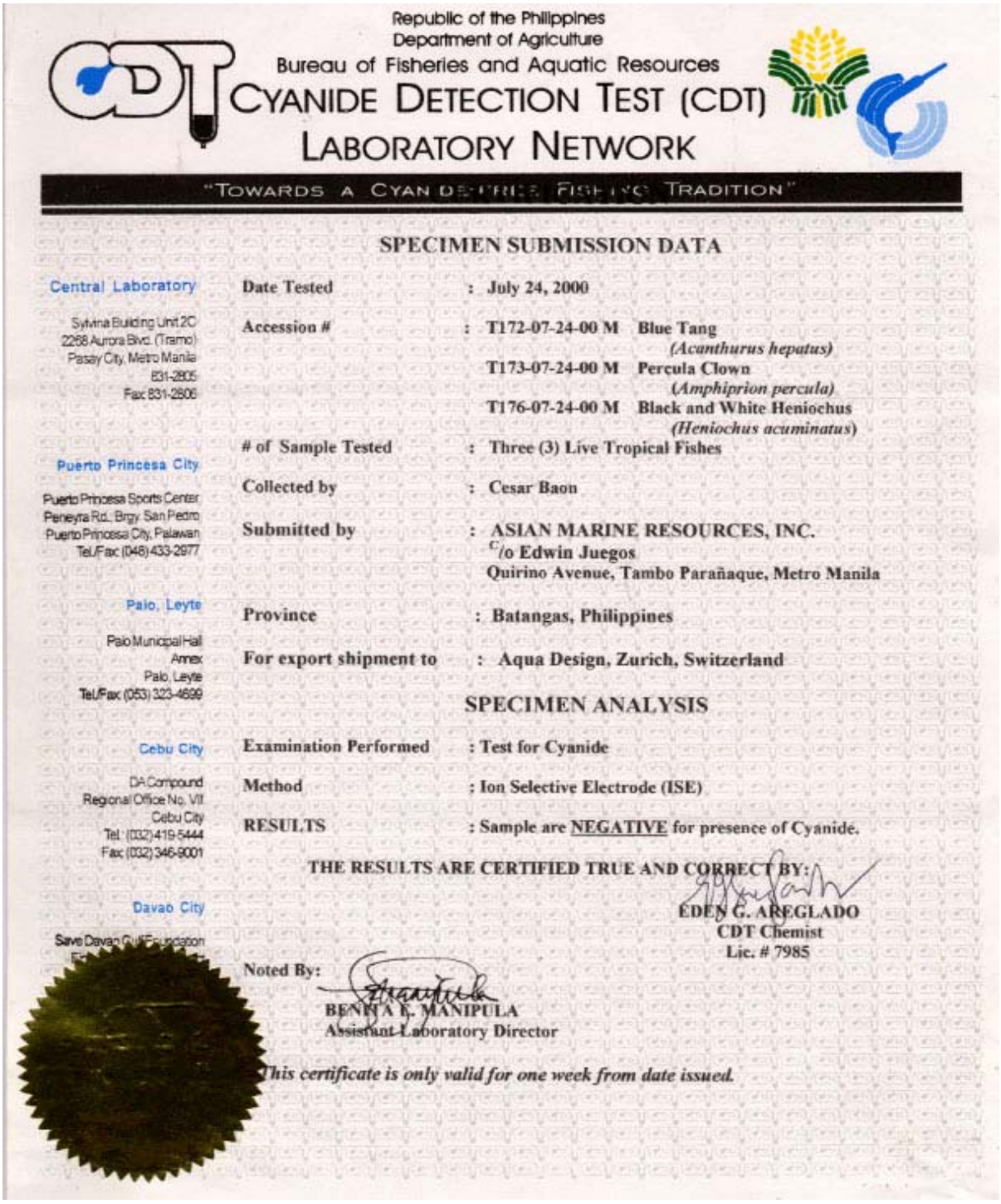
EMPLOYEES PROFILE - CDT LABORATORY NETWORK

Name	Position Held	Date Hired	Undergraduate Degree	Post Graduate Degree or Trainings	Licensure	Lic. #
Central Lab.						
Anita Puño	Laboratory Manager	Oct-98	B.S. Food & Nutrition	*M.S. Food Tech	na	na
Herminia O. Carandang	Chemist I	May-00	B.S. Chemistry		PRC Passer	8101
Eden G. Areglado	Chemist II	Sep-99	B.S. Chemistry/Sup. BSBiology	*M.S. Chemistry	PRC Passer	7986
Benita E. Manipula	Asst. Lab. Director/Chemist	Jan-94	B.S. Chemistry	*M.S Chem, *M.M, *M.O	PRC Passer	6963
Bega B. Musa	Records Administrator	May-95	B.S. Medical Tech/BSEEd- units		PRC Passer	598488
Haide Trono	Fisheries Officer 1	Mar-98	B.S. Fisheries	*M.S Envi Chem.	CSC Passer	
Ana O. Dumdumaya	Laboratory Tech.	Jun-99	B.S. Educ (undergrad.)		na	na
Majilia E. Cuenca	Laboratory Tech.	Jun-01	B.S. Computer Eng'g.		Eng'g.	ok
Eric G. Parfan	Fisheries Officer II	Mar-98	B.S. Fisheries	*M.S. Envi. Science	na	Fish Warden
Joy C. Alban	MIS Director/Fish. Officer III	Oct-94	B.S. Fisheries	*P.B. computer Technol	CSC passer	Fish Warden
Gemma C. Mose	Chemist I		B.S. Chemistry			
Madonna Villar	Chemist I		B.S. Chemistry			
Jocelyn Jocson	Chemist I		B.S. Chemistry			
Melissa Melchor	Chemist I		B.S. Chemistry			
Robert Pimentel	Chemist I		B.S. Chemistry			
Pedro Joel Resuello	Laboratory Manager	Resigned	B.S. Nursing	PRC Passer		
Cebu Lab.						
Glenn G. Tuazon	Chemist I	Mar-99	B.S. Chemistry		PRC Passer	
Aimie Reboles	Lab. Tech	Jun-98	B.S. Commerce		na	
Naije Tan	Chemist I		B.S. Chemistry			
Cheryl Arbon	Chemist I	Resigned	B.S. Chemistry	PRC Passer		
Imelda Almaden	Lab. Tech	May-97			PRC Passer	28739
Shahlom Christian Alinsug	Fisheries Officer	Jun-98			na	Fish Warden
Palo Lab.						
Pablo G. Gonzales	Chemist I	Dec-98	B.S. Chemistry		na	8025
Estanislao O. Cabreros	Fisheries Officer	May-97	B.S Architecture		na	Fish Warden
Ediberto Navarrosa	Utility/Messenger	May-97	Civil Technology (undergraduate)		na	Fish Warden
Timmy Villas	Fish Biologist	May-97	B.S Agricultural Technology		na	Fish Warden
Nadit Acedillo	Lab. Tech	Resigned	B.S. Nursing	PRC Passer		
Conrado Orollo	Fisheries Officer		B.S. Industrial Eng'g.			Fish Warden

Appendix E List of Employees and Qualifications (part B)

Zamboanga Lab.							
Nhilda M Astillero	Chemist I	May-95	B.S. Chemistry	MAT- Chemistry	PRC Passer	969	
Ma. Eden Alvarez	Chemist I	Jan-98	B.S. Chemistry		PRC Passer	7847	
Glenda A. Tabar	Lab. Tech	Aug-94	B.S. Chemistry	*M.S. Chemistry	CSC Passer		
Rachel Tenorio	Lab Tech	Jun-96	B.S. Chemistry		na		
Mark Anthony Bustamante	Utility	Mar-99	College (undergrad.)		na		
Ma. Ruby Nazario	Laboratory Manager	Jul-95	B.S. Commerce		na		
Palawan Lab.							
Emma R. Sutplido	Chemist II	Aug-93	B.S. Chemistry	MAT-Chem, MPA	PRC Passer	2958	
Gemma M. Santiago	Laboratory Manager	Aug-93	B.S. Biology		na		
Robert Gonzales	Office Assistant	Jun-98	B.S. Fisheries		CSC Passer	Fish Warden	
Manuel Almares	Fisheries Officer	Resigned					
Fred Taladua	Fisheries Officer	May-95	B.S. Educ				
Anabelle Vitero	Lab. Tech	Resigned					
Marie Fe V	Lab,, Tech						
Loida S. Japson	Chemist I	May-97	B.S. Chemistry	MAT-Chem.	PRC Passer	6605	
Geraldine Enofre	Lab. Tech	Jun-01	Computer Sys.Design & Prog'g				
Davao Lab.							
Perlina L. Escribir	Chemist I	Mar-98	B.S. Chemistry		PRC Passer	7838	
Albert Cahilog	Laboratory Manager	Dec-98	AB English				
Jennifer Into	Lab. Tech	Mar-98	BS Chemical Engineering				

Appendix F Certificates Issued By the BFAR/IMA CDT Laboratories



Republic of the Philippines
Department of Agriculture
Bureau of Fisheries and Aquatic Resources

CYANIDE DETECTION TEST (CDT) LABORATORY NETWORK

"TOWARDS A CYANIDE FREE FISHING TRADITION"

SPECIMEN SUBMISSION DATA

Central Laboratory
Sylvina Building Unit 2C
2268 Aurora Blvd. (Tramo)
Pasay City, Metro Manila
831-2805
Fax 831-2806

Puerto Princesa City
Puerto Princesa Sports Center
Peneyra Rd., Brgy. San Pedro
Puerto Princesa City, Palawan
Tel./Fax (048) 433-2977

Palo, Leyte
Palo Municipal Hall
Annex
Palo, Leyte
Tel./Fax (053) 323-4699

Cebu City
DA Compound
Regional Office No. VII
Cebu City
Tel.: (032) 419-5444
Fax (032) 346-9001

Davao City
Save Davao Gulf Foundation

Date Tested	:	July 24, 2000
Accession #	:	T172-07-24-00 M Blue Tang *(Acanthurus hepatus)* T173-07-24-00 M Percula Clown *(Amphiprion percula)* T176-07-24-00 M Black and White Heniochus *(Heniochus acuminatus)*
# of Sample Tested	:	Three (3) Live Tropical Fishes
Collected by	:	Cesar Baon
Submitted by	:	ASIAN MARINE RESOURCES, INC. c/o Edwin Juegos Quirino Avenue, Tambo Parañaque, Metro Manila
Province	:	Batangas, Philippines
For export shipment to	:	Aqua Design, Zurich, Switzerland

SPECIMEN ANALYSIS

Examination Performed	:	Test for Cyanide
Method	:	Ion Selective Electrode (ISE)
RESULTS	:	Sample are NEGATIVE for presence of Cyanide.

THE RESULTS ARE CERTIFIED TRUE AND CORRECT BY:

EDEN G. AREGLADO
CDT Chemist
Lic. # 7985

Noted By:

BENITA E. MANIPULA
Assistant Laboratory Director

This certificate is only valid for one week from date issued.

Appendix G - Schematic overview of the cyanide testing process