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DEFRApH - Sample collection and handling procedures

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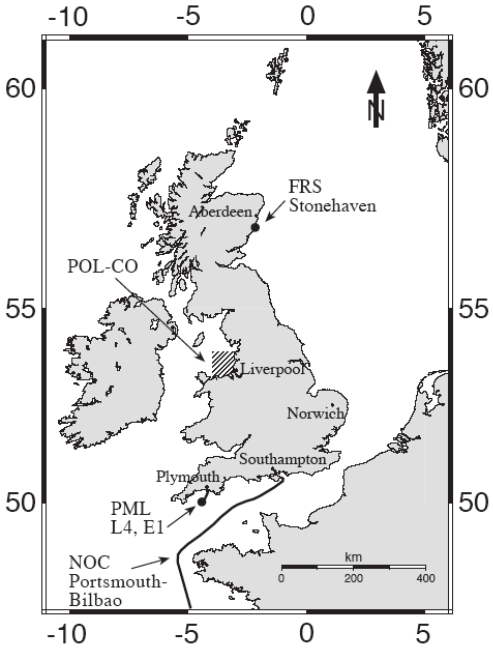
Frontispiece

Denise Cummings PML sampling for the DEFRApH project at the "E1" station



Map of the DEFRApH sampling locations

DOCUMENT DATA SHEET

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DEFRApH - Sample collection and handing procedures: DEFRApH - A study to define the present state of UK waters with respect to potential changes in the degree of acidification (pH) of these waters; combining collection of new data over two years, review of pre-existing information and numerical analysis. UKMMAS Monitoring Handbook Entry

REFERENCE
Southampton, UK: National Oceanography Centre, Southampton, 20pp.
(National Oceanography Centre Internal Document, No. 02)
(Unpublished manuscript)

ABSTRACT

All chemical and biogeochemical process in the sea are affected by the acidity of the water. Acidity is therefore fundamental property of seawater. The growing concern that the acidity of the oceans might be increasing has revealed weaknesses in our knowledge of this fundamental property and its variation in space and time. In 2008 the DEFRApH project (DEFRA contract ME4133) was initiated to provide this missing information in UK related waters. It required sampling for and analysis of the total inorganic carbon and total alkalinity content of samples. This reports documents the procedures sued for sampling. A companion document Hartman Dumousseaud and Roberts (NOC Internal Document No. 01) describes in detail the analytical procedures used and the calculation of the results.

KEYWORDS
Mercuric Chloride, Preservation, Safety, Sample collection, Total alkalinity, Total inorganic carbon, Transportation

ISSUING ORGANISATION

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Contents

Section 1

Internationally agreed procedures for water sampling for the parameters of the oceanic carbon dioxide system

Taken from
Version 3.0 Standard Operating Procedure SOP 1 — Water sampling October 12, 2007
Guide to Best Practices for Ocean CO2 Measurements PICES Special Publication 3
IOCCP Report No. 8: A. G. Dickson, C. L. Sabine & J. R. Christian

1. Scope and field of application
This SOP describes how to collect discrete samples, from a Niskin or other water sampler, that are suitable for the analysis of the four measurable inorganic carbon parameters: total dissolved inorganic carbon, total alkalinity, pH and CO_2 fugacity.

2. Principle
A sample of sea water is collected in a clean glass container in a manner designed to minimize gas exchange with the atmosphere (note: CO_2 exchange affects the various carbon parameters to differing degrees ranging from the very sensitive CO_2 fugacity, $f(CO_2)$, to alkalinity which is not affected by gas exchange). The sample may be treated with a mercuric chloride solution to prevent biological activity, and then the container is closed to prevent exchange of carbon dioxide or water vapour with the atmosphere.

3. Apparatus
The sample containers are somewhat different depending on which parameter is being collected, but the basic concept is similar for the four possible inorganic carbon samples. In general, one needs a flexible plastic drawing tube, a clean. glass sample container with stoppers, a container and dispenser for the mercuric chloride solution and a sampling log to record when and where each of the samples were collected. (Cleaning sample containers by pre-combustion in a muffle furnace will remove any organic carbon and associated micro-organisms. Some groups soak the bottles in 1 N HCl; however, care must be taken to remove all residual acid during rinsing.)

3.1 Drawing tube
Tygon® tubing is normally used to transfer the sample from the Niskin to the sample container; however, if dissolved organic carbon samples are being collected from the same Niskins, then it may be necessary to use silicone tubing to prevent contamination from the Tygon®. The drawing tube can be pre-treated by soaking in clean sea water for at least one day. This minimizes the amount of bubble formation in the tube when drawing a sample.

3.2 Sample container
The sample container depends on the parameter being measured. Typically, the $f(CO_2)$ samples are analyzed directly from the sample container so they are collected in 500 cm^3 volumetric flasks that have been pre-calibrated for a documented volume and sealed with screw caps that have internal plastic conical liners. Samples for pH are also typically analyzed directly from the sample containers. For spectrophotometric pH measurements, the samples are collected directly into 10 cm path-length optical cells and sealed with polytetra-fluoroethylene (Teflon®) caps ensuring that there is no headspace. For CT and AT, high quality borosilicate glass bottles, such as Schott Duran, are recommended for both temporary and long-term storage. The bottles should be sealed using greased ground glass stoppers held in place with some form of positive closure, or in some alternate gas-tight fashion.

3.3 Mercury dispenser

The $f(CO_2)$ and CT samples should be poisoned with a mercuric chloride solution at the time of sampling. The AT samples have historically been poisoned as well, but tests have suggested that poisoning may not be required if open ocean samples are kept in the dark at room temperature and are analyzed within 12 hours. Samples for pH are typically not poisoned because the sample size is relatively small and the samples are usually analyzed very quickly after sampling. Although any appropriately sized Eppendorf type pipette can be used to add the mercuric chloride solution, it may be more convenient to use a re-pipetter that can be mounted near the sample collection area. All equipment should be properly labelled for safety.

4. Reagents

4.1 Mercuric chloride solution

Samples collected for $f(CO_2)$, CT, and, in some cases, AT, should be poisoned with a mercuric chloride solution to stop biological activity from altering the carbon distributions in the sample container before analysis. A typical solution is saturated mercuric chloride in deionised water. However, saturated solutions have been known to clog the pipette in very cold weather, so some investigators use twice the volume of a 50% saturated solution. Standard volumes used for saturated solutions are 0.05–0.02 % of the total sample volume.

4.2 Stopper grease

CT and AT samples are typically collected in borosilicate glass bottles with ground glass stoppers. To form an airtight seal, the stoppers should be greased. Apiezon® L grease has been found to be suitable for this purpose; other greases may also work. Care should be taken not to transfer the grease onto the Niskin bottle as this could interfere with other analyses.

5. Procedure

5.1 Introduction

Collection of water at sea from the Niskin bottle (or other sampler) must be done soon after opening the sampler and before much other water has been removed from it. This is necessary to minimize exchange of CO_2 with the air space in the sampler which affects all carbon parameters except AT. Other gas samples (e.g., He, CFCs, O_2) have faster exchange rates than CO_2 and are usually sampled before carbon, but it is desirable that the carbon samples be collected before the Niskin bottle is half empty and within 10 minutes of it being first opened. A typical sampling order for carbon is f(CO2), pH, CT, then AT.

5.2 Filling procedure

Rinse the sample bottle — If the bottle is not already clean, rinse it twice with 30–50 cm^3 of sample to remove any traces of a previous sample.

Fill the sample bottle — Fill the bottle smoothly from the bottom using a drawing tube, which extends from the Niskin drain to the bottom of the glass sample bottle. For f(CO2), pH, and CT, it is critical to remove any bubbles from the draw tube before filling. Overflow the water by at least a half, and preferably by a full, bottle volume (The amount of overflow water can be estimated by measuring how long it takes to fill a sample bottle, and allowing the water to flow for a period of 1.5 times that.)

Adjust the headspace — A headspace of 1% of the bottle volume is left to allow for water expansion (see Annexe to this procedure), i.e., 2.5 cm^3 for a 250 cm^3 bottle. This can be achieved by pinching off the draw tube before removing it from the sample bottle or removing excess water using a plastic pipette with a bulb. pH samples should not have a headspace.

Add mercuric chloride — Mercuric chloride is added to poison the sample; the recommended minimum amount is about 0.02% by volume of a saturated aqueous solution. Thus to poison a 250 cm^3 sample requires 0.05 cm^3 (50 µl) of saturated mercuric chloride (or 0.10 cm^3 of a 50% saturated solution). Maximum amount is 0.1% by volume of a saturated aqueous solution, or a smaller percentage than measurement precision of CT and $f(CO_2)$.
Close the bottle — Seal the bottle carefully to ensure that it remains gas-tight. If it is to be sealed using a greased ground glass stopper, first wipe the excess water from the ground glass in the bottle neck, then insert the stopper completely, and finally twist the stopper to squeeze the air out of the grease to make a good seal.
Finally, secure the lid — Use a rubber band or other positive closure, then invert the bottle several times to disperse the mercuric chloride solution thoroughly.
The recommended procedure for re-greasing (or greasing) a stopper is as follows:
(a) wipe the stopper with a tissue to remove as much grease as possible and (b) grease the stopper with 4 strips of grease, each strip extending two thirds of the way from the top towards the bottom of the ground portion of the stopper. This provides a path for air to escape when the stopper is inserted into the neck of the bottle.

5.3 Sample storage

The samples should be stored in a cool, dark, location (preferably refrigerated but not frozen) until use.

5.4 Sample documentation

The following information must be recorded in the sampling logbook at the time of sampling:

- Time and date when taken;
- Full name of person who took sample;
- Location: an unambiguous designation of the station, cast, and bottle number from which the sample was taken;
- Container designation: a number or alphanumeric symbol unique to the sample container;
- Comments: additional information such as conditions when sampling, problems with sample collection, etc.

6. Quality assurance

Some duplicate sampling is recommended, both from the same sampler (e.g., Niskin bottle) and, if possible, from two samplers tripped together at the same depth, to assess the quality of the sampling procedures.

Annexe

How large a headspace should be left in a sample bottle?
The volume of the headspace is chosen so as to leave room for expansion of the sea water on warming, while being sufficiently small to minimize the amount of gas exchange between the headspace and the bulk of the sample. The closure system must be adequate to retain the pressure exerted by the expansion.
The apparent change in the volume of a fixed mass of sea water can be calculated by allowing for the change in the density of the sea water and the expansion of the glass container. The total change over the temperature range 0–40 °C is about 1 %. (The effect of expansion on the volume of the borosilicate glass bottle is only 0.04 % over this range.) One third of this expansion occurs on heating the sea water from 0 to 20 °C, the remaining two thirds on heating it from 20 to 40 °C.
The pressure in the headspace of a container heated from a temperature *t1* to *t2* can be estimated, allowing for the following:

• the expansion of the sea water in the bottle,
• the change in solubility of gases such as N_2, O_2, Ar,
• the thermal expansion of the gas phase,
• the change in the vapour pressure of H_2O in the gas phase.

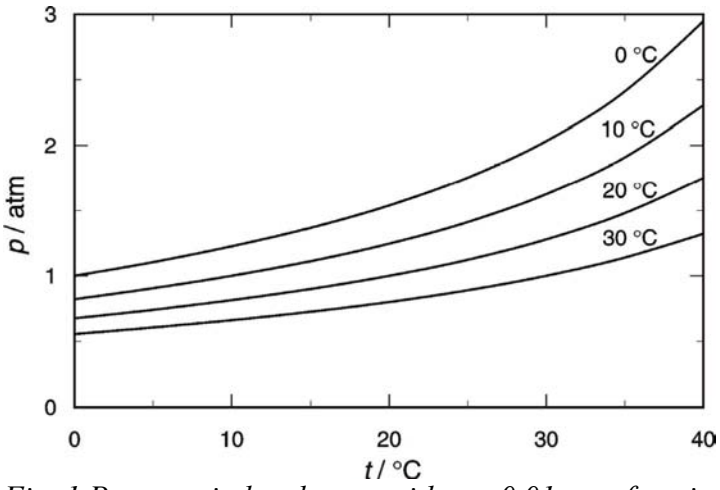


Fig. 1 Pressure in headspace with r = 0.01 as a function of temperature for various initial temperatures. In calculating this, it was assumed that the gases—N_2, O_2, Ar, H_2O—were initially at solubility equilibrium with sea water at the starting temperature, that they behaved ideally and that the initial pressure in the bottle at the indicated temperature was 1 atm.

Defining the initial headspace ratio,

$$r = V_{(headspace)}/V_{(sea\ water)}\ , (1)$$

allows the calculation of the approximate pressure in the headspace of a closed container as a function of temperature. Clearly, if cold samples (< 10 °C) are likely to be heated above 30 °C, there is a risk of them leaking if the headspace ratio is significantly less than 1 %. There is, however, an additional factor to be taken into account when determining the optimal headspace size: gas exchange with the headspace. The change in total dissolved inorganic carbon (ΔCT) resulting from this gas exchange can be derived from mass balance considerations:

$$\Delta CT = \{(p(CO_2)*V/T)_{initial} - (p(CO_2)*V/T)_{final}\} / R*m_{(sample)}$$

where $p(CO_2)$ is the partial pressure of CO_2 in a headspace of volume V and at a temperature T corresponding to the initial condition (when the bottle is closed) and the final condition (when the bottle is analyzed), R is the gas constant and m(sample) is the mass of the sample. The volume of the headspace decreases as the contents heat up (due to the expansion of the sea water) partially compensating for the decrease in the solubility of the various gases, thus the greatest loss of CO_2 will occur if the sea water has a high $p(CO_2)$ but does not warm up significantly in the container. Even then, provided that the headspace ratio is less than 0.01, ΔCT will be less than 0.5 μmol kg^{-1}.
Gain or loss of CO_2 gas is not significant when collecting discrete samples for alkalinity measurement; however, if p(CO2) is to be measured, the so-called "buffer factor" comes into

play and the resultant relative error in p(CO2) is approximately 10 times that in CT, i.e., for a change of –0.5 μmol kg^{-1} in CT, the change in p(CO2) is about –0.25 %. This corresponds to a change in pH of about +0.001.
A headspace of 1 % is thus optimal for the collection of CO_2 samples, provided that they will not be exposed to temperature changes of 30°C or more. If this cannot be assured, it is preferable to allow a larger headspace and to estimate the appropriate correction.

Section 2

NOCS (and DEFRApH) procedures for Collection and preservation of samples of sea water prior to being stored before analysis to determine alkalinity and total CO2 content of sample.

Description of equipment

Plastic bottle containing 40ml of saturated solution of Mercuric Chloride (7g /100ml)
Finpipette automatic pipette 50 micro litres (0.050 ml)
Eppendorf automatic pipette 2.5 ml
Glass bottles containing seawater sample (250 ml)
Silicon grease
PVC tape
Marker pen
Log sheet
Bucket for transport of sample bottle
Pencil

Procedure

Collect equipment.
Take next sample bottle from red and grey plastic transport box
Check ground glass stopper has been lightly smeared with silicon grease.

<u>At sampling point</u>

1. Remove stopper from glass bottle
2. Use silicon grease to light grease ground glass neck of bottle
3. Record date and time on **log sheet and on bottle label**.
4. Flush tap by quarter filling bucket
5. Rinse bottle by half filling bottle and shaking vigorously
6. Empty
7. Repeat rinse
8. Insert plastic tube on sampling tap to base of bottle.
9. Slowly fill bottle and first inverting it then turn upright. Slowly rotate bottle as it fills and making sure no bubbles collect in side the bottle.
10. Allow the bottle to over flow by the volume of the bottle
11. Slowly remove the tube from the bottle.
12. Insert the stopper
13. Remove stopper from the glass bottle.
14. Using 2.5ml pipette to withdraw **<u>2.5 ml</u>** of seawater from the bottle
15. Replace stopper

<u>In stable work area</u>

1. WORK WITH CLEAN HANDS
2. Lay out bits needed for adding mercuric chloride to sample
3. Pipette, pipette tips, mercuric chloride bottle, PVC tape
4. In case of spill have ready rubber gloves, paper towel and plastic bag
5. Put a tip on the microlitre pipette.

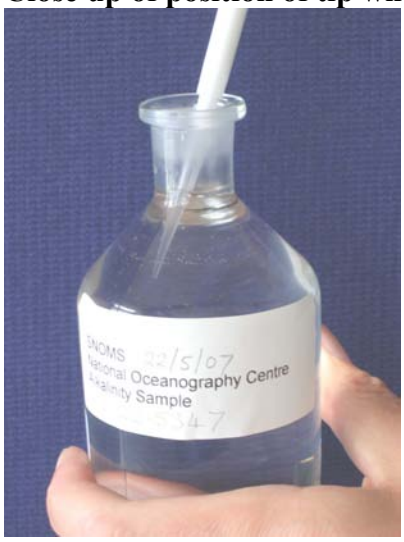
6. Pipette 50 microlitre of mercuric chloride solution in the seawater sample. Place tip of pipette about half the length of the pipette tip below the surface of the seawater to do this.
7. Rinse pipette by taking up and immediately ejecting back into the bottle some seawater from the top of the bottle.
8. Put down pipette
9. Reseal the bottle of mercuric chloride securely.
10. Eject the tip of the pipette into a plastic bag.
11. Replace the stopper in the glass bottle.
12. Bind the stopper in to the bottle using 3 loops of tightly stretched PVC tape round the bottle.
13. Replace the bottle in the storage crate.
14. Check the log sheet.

Note on use of Mercuric Chloride
Mercuric Chloride is toxic. At the concentration used it might cause skin irritation or burns if prolonged contact with the skin occurs.
Best practice is to work with CLEAN HANDS and to wash them immediately a splash should occur FIRST having closed the bottle securely.
If spill occurs: (1) if hands are contaminated wash them first (2) put on the rubber gloves provided (2) dry up the spill with dry towel paper (3) place contaminated towel in a plastic bag (4) wipe the splashed area with damp water towel (5) add this damp towel to the plastic bag. (6) remove the rubber gloves and add them to the bag (7) seal the bag (8) estimate the amount spilt – the bottle started with about 40 of a solution of 7g in 100ml. (9) label the bottle with the **amount** (**XX mls of 7%HgCl2 solution**) and **MERCURIC CHLORIDE Hazchem 2X, UN 1624.** (10) Store in a safe place for disposal on shore.

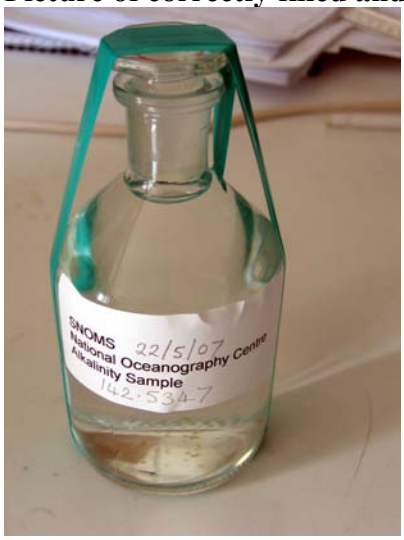
Note on use of microlitre pipettes
Microlitre pipettes are high precision pieces of scientific equipment when used correctly. They are used with disposable tips that are the only part of them that contacts the solution being pipetted.
A new tip should be used each time the pipette is used.
It should be pushed firmly on the pipette and discarded directly into a plastic disposal bag after use.
The button in the end of pipetted is used for filling and discharging the pipette. The button can be pushed in two distances. To fill the pipette the first stop is used. To empty the pipette the button is pushed in further to the second stop.
When filling the pipette the button should be pushed in and then the tip of the pipette placed about 5mm below the surface of the liquid being collected. The button should then be released slowly.
To dispense the solution the tip of pipette should be about half the length of the pipette tip below the surface of the seawater. The button should then be pushed on all the way. The tip should then be removed from the seawater **before** the button is released. The tip should then be ejected in a plastic waste bag.

Picture of pipette being used correctly

Close up of position of tip when injecting $HgCl_2$ solution



Picture of correctly filled and labelled bottle



Section 3

Example Risk Assessment:
Collection and preservation of samples of sea water prior to being stored before analysis to determine total dissolved inorganic carbon and total alkalinity content of sample.

Reference Pride of Bilbao
Date of assessment 10/09/08

Name of Assessor David Hydes
National Oceanography Centre
Southampton UK
e-mail djh@noc.soton.ac.uk
phone + 44 23 8059 65347

2 Subject of Assessment
Collection and preservation of samples of sea water prior to being stored before analysis to determine alkalinity content of sample.
3. Person responsible for equipment
All involved in sampling during Pride of Bilbao surveys
4. Description of equipment
Plastic bottle containing 40ml of saturated solution of Mercuric Chloride (7g /100ml)
Finpipette automatic pipette 50 micro litres (0.050 cc)
Glass bottles containing seawater sample (250 cc)
Silicon grease
PVC tape
Marker pen
Log sheet
Bucket for transport of sample bottle
5. What are the main tasks involved in this work activity?
In engine room collection of water sample.
In stable clean area pipetting 50 microlitres of mercuric chloride into each sample bottle.
Sealing sand storage of bottle
6. What machinery, equipment and personnel are involved?
The person doing the work
7. Are any COSHH regulated substances involved?
Mercuric Chloride (Hazchem 2X). Data sheet attached
8. What environmental constraints are there, if any?
Work should be done in stable environment. All bottles should be secure against being dislodged by ship movement
9. What can go wrong?
Chemical can be spilt. Glass bottles can break.
10. Are there any other aspects you should consider?
Clean hands should be handling the mercuric chloride bottle. Hands should be washed as soon a practical if splashed using soap and plenty of water. Rubber gloves should be worn when wiping up any spillage. Boiler suit should also be worn. If the saturated Mercuric

Chloride is split it should be wiped up with paper towel. The wet towel should be stored in a labelled plastic bag and disposed of ashore.

Signed

Section 4

Material Safety Data Sheet Mercury(II) chloride

Mercuric Chloride
Saturated Solution
(7g / 100ml)
Hazchem 2X(1)
UN 1624

MSDS Name:
Mercury(II) chloride
Source Company Identification:Fisher Scientific UK Bishop Meadow Road, Loughborough Leics. LE11 5RG For information in Europe, call:(01509) 231166 Emergency Number, Europe: 01509 231166
Composition, Information on Ingredients
CAS#: 7487-94-7 Chemical Name: Mercury(II) chloride %: >99.5
EMERGENCY OVERVIEW Very toxic if swallowed. (A dose of 1 gram can be fatal to humans if swallowed.) Causes burns. Danger of serious damage to health by prolonged exposure in contact with skin. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Light sensitive. Spills/Leaks:
Clean up spills immediately, observing precautions in the Protective Equipment section.
Handling and Storage Handling:
Wash thoroughly after handling. Wash hands before eating. Remove contaminated clothing and wash before reuse. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. Extreme care should always be taken to prevent skin and gastrointestinal absorption because these routes of entry can greatly increase the total body burden.
Storage:
Store in a tightly closed container. Keep away from food and drinking water. Store in a cool, dry, well-ventilated area away from incompatible substances. Store protected from light.
Potential Health Effects Eye:
Exposure to mercury or mercury compounds can cause discoloration on the front surface of the lens, which does not interfere with vision. Causes severe eye irritation and possible burns. Contact with mercury or mercury compounds can cause ulceration of the conjunctiva and cornea.

Skin:
May be fatal if absorbed through the skin. Causes severe skin irritation and possible burns. May cause allergic contact dermatitis.
Ingestion:
May be fatal if swallowed. Causes gastrointestinal irritation with nausea, vomiting and diarrhea. Causes gastrointestinal tract burns. May cause muscle tremor and impaired motor function. May cause cardiac disturbances.
Inhalation:
May cause central nervous system effects including vertigo, anxiety, depression, muscle incoordination, and emotional instability. May cause gastrointestinal effects including gum and mouth inflammation, jaw necrosis, and loosening of the teeth. May cause burns to the respiratory tract. Acute exposure to high concentrations of mercury vapors may cause severe respiratory tract irritation.
Chronic:
Prolonged or repeated skin contact may cause dermatitis. Chronic inhalation and ingestion may cause effects similar to those of acute inhalation and ingestion. May cause reproductive and fetal effects. Chronic ingestion may cause accumulation of mercury in body tissues. Laboratory experiments have resulted in mutagenic effects. May be rapidly transferred across the placenta and cause adverse fetal effects.
First Aid Measures Eyes:
In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical aid immediately.
Skin:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse.
Ingestion: POISON material. If swallowed, get medical aid immediately. Only induce vomiting if directed to do so by medical personnel. Never give anything by mouth to an unconscious person.
Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
Notes to Physician:
The concentration of mercury in whole blood is a reasonable measure of the body-burden of mercury and thus is used for monitoring purposes. Persons with kidney disease, chronic respiratory disease, liver disease, or skin disease may be at increased risk from exposure to this substance.
Antidote:
The use of Dimercaprol or BAL (British Anti-Lewisite) as a chelating agent should be determined by qualified medical personnel. The use of d-Penicillamine as a chelating agent should be determined by qualified medical personnel.

Exposure Controls, Personal Protection
Exposure Limits CAS# 7487-94-7: United States OSHA: 0.1 mg/m3 Ceiling (Mercury, aryl and inorganic compounds). Malaysia: (mercury, aryl and inorganic compounds): 0.1 mg/m3 TWA (as Hg)

Personal Protective Equipment Eyes:
Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin:
Wear appropriate protective gloves to prevent skin exposure.

Clothing:
Wear appropriate protective clothing to prevent skin exposure.
Section 9 - Physical and Chemical Properties

Physical State:	Crystals
Color:	white
Odor:	odorless
pH:	4.7
Vapor Pressure:	slightly volatile @RT
Viscosity:	Not applicable.
Boiling Point:	300 deg C (572.00F)

Freezing/Melting Point: 277 deg C (530.60F) Autoignition Temperature: Not available. Flash Point: Not applicable. Explosion Limits: Lower:Not available Explosion Limits: Upper:Not available Decomposition Temperature: Not available Solubility in water: Soluble Specific Gravity/Density: 5.44 at 25C Molecular Formula: HgCl2 Molecular Weight: 271.50
Stability and Reactivity
Chemical Stability:
Stable at room temperature in closed containers under normal storage and handling conditions.
Incompatibilities with Other Materials

Disposal Considerations
Products considered hazardous for supply are classified as Special Waste and the disposal of such chemicals is covered by regulations which may vary according to location. Contact a specialist disposal company or the local authority or advice. Empty containers must be decontaminated before returning for recycling.

Section 5 - Transport and Regulatory Code Information

Transport

IATA

Shipping Name:	MERCURIC CHLORIDE
Hazard Class:	6.1
UN Number:	1624
Packing Group:	II

IMO

Shipping Name:	MERCURIC CHLORIDE
Hazard Class:	6.1
UN Number:	1624
Packing Group:	II

RID/ADR

Shipping Name:	MERCURIC CHLORIDE
Hazard Class:	6.1
UN Number:	1624
Packing Group:	II

Regulatory codes

European/International Regulations European Labelling in Accordance with EC Directives
Hazard Symbols: T+ C N Risk Phrases:
R 28 Very toxic if swallowed.
R 34 Causes burns.
R 48/24/25 Toxic : danger of serious damage to
health by prolonged exposure in contact with skin and
if swallowed.
R 50/53 Very toxic to aquatic organisms, may cause
long-term adverse effects in the aquatic environment.

Safety Phrases: S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection. S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S 60 This material and its container must be disposed of as hazardous waste. S 61 Avoid release to the environment. Refer to special instructions/safety data sheets.