EPA-909/9-74-004

WORKSHOP

ON

SAMPLING, MONITORING AND ANALYSIS

OF

WATER AND WASTEWATER

March 6-12, 1974
Honolulu, Hawaii

U.S. Environmental Protection Agency, Region IX
San Francisco CA 94111
June 1974

EPA-909/9-74-004

File

WORKSHOP

ON

SAMPLING, MONITORING AND ANALYSIS

OF

WATER AND WASTEWATER

March 6-12, 1974
Honolulu, Hawaii

U.S. Environmental Protection Agency, Region IX
San Francisco CA 94111
June 1974

INTRODUCTION

On March 6-12, 1974 the U.S. Environmental Protection Agency conducted a Workshop on Sampling, Monitoring and Analysis of Water and Wastewater. The training course was sponsored by the Environmental Protection Agency, Region IX in cooperation with the National Field Investigation Center (Denver, Colorado), and the State of Hawaii Department of Health, the University of Hawaii, and the Hawaii Water Pollution Control Association.

Over 85 persons attended the 5-day training course which was held on the campus of the University of Hawaii. Attendees included: State of Hawaii public health officials, State and local laboratory and field personnel as well as technical personnel from American Samoa, consulting engineers, and University of Hawaii students.

The workshop provided information and training in both the standard and new techniques currently employed in the sampling, monitoring and analysis of water and wastewater.

This document is a compilation of papers presented during that workshop. Also included is a list of the several publications which were disseminated as part of the training course. In most cases, copies can be obtained through the Government Printing Office.

CONTENTS

WORKSHOP ON
SAMPLING, MONITORING AND ANALYSIS OF WATER AND WASTEWATER

March 6-12, 1974

SPEAKERS

Paul DeFalco, Jr. Administrator	EPA, Region IX San Francisco CA 94111
Henri P. Minette Deputy Director of Health	State of Hawaii Dept. of Health Honolulu HI 96801
Ho Young Chemist	EPA. Region IX San Francisco CA 94111
Kathleen Shimmin Microbiologist	EPA, Region IX San Francisco CA 94111
Milton Tunzi Biologist	EPA, Region IX San Francisco CA 94111
Helen Johnson Biological Aid	EPA, Region IX San Francisco CA 94111
Carroll Wills Enforcement Specialist	EPA, Nat'l Field Investigation Ctr. Denver CO 80225
James Hathaway Sanitary Engineer	EPA, Nat'l Field Inventigation Ctr. Denver CO 80225
Laurence Walz Physical Science Technician	EPA, Nat'l Field Investigation Ctr. Denver CO 80225
James Kumagai Sanitary Engineer	Sunn, Low, Tom and Hara, Inc. Honolulu, HI 96813

WORKSHOP ON
SAMPLING, MONITORING AND ANALYSIS OF WATER AND WASTEWATER

March 6-12, 1974

AGENDA

March 6, 1974	Wednesday
8:30	Registration
9:00	Introduction (DeFalco, Minette)
	Federal Requirements for Sampling, Sample Handling, Analyses, Quality Assurance, WQ Act (Shimmin)
10:00	Coffee Break
10:15	Survey Planning - Site Selection (Tunzi)
12:00	Lunch
1:30	Parameter Selection (Tunzi)
2:15	Coffee Break
2:30	Monitoring and Flow Measurement (Hathaway and Walz)
3:30	Compliance Monitoring (Wills)
3:45-4:45	Comparison of Sampling Methods in Waste Effluents (Kumagai)
March 7, 1974	Thursday
8:30	Monitoring and Flow Measurement (Hathaway and Walz)
10:00	Coffee Break
10:15	Instrumentation (Walz)
12:00	Lunch
1:30-5:00	Field Exercise (Johnson, Shimmin, Tunzi)
March 8, 1974	Friday
8:30	Discussion of Field Exercise -questions
9:00	Chemistry (Young) Federal Register-304 g Guidelines
10:15	Coffee Break
10:30	Bacteriology (Shimmin) Quality Assurance
12:00	Lunch

AGENDA - Continued

March 8, 1974	Friday (continued)
1:30	Statistics
2:30	Coffee Break
2:45-4:30	Biostimulation, Toxicity (Tunzi)
March 11, 1974	Monday (LABORATORY)
8:30	Registration (New Attendees)
9:00	Introduction, Federal Requirements (Shimmin)
9:30	Bacteriology (Shimmin, Johnson)
12:00	Lunch
1:30	Chemistry (Young) Demand, Nutrient, Oil and Grease
3:00	Coffee Break
3:15-5:00	Chemistry (Young) Demand, Nutrient, Oil and Grease
March 12, 1974	Tuesday
8:00	Heavy Metals (Young) Pesticides
10:00	Coffee Break
10:15	Bioassays (Tunzi)
12:00	Lunch
1:30	Biological Methods Manual (Tunzi)
2:30	Coffee Break
2:45-5:00	Quality Assurance (Shimmin)

U.S. ENVIRONMENTAL PROTECTION AGENCY
IN COOPERATION WITH
THE STATE OF HAWAII DEPARTMENT OF HEALTH, THE UNIVERSITY OF HAWAII
AND THE HAWAII WATER POLLUTION CONTROL ASSOCIATION

Workshop on Sampling, Monitoring, and Analysis
of Water and Wastewater

March 6,7,8,11,12, 1974

REGISTRATION FORM

Name: _____

Address: _____

_____

Employer: _____

Address: _____

_____

Occupation Title: _____

WORKSHOP ON
SAMPLING, MONITORING AND ANALYSIS OF WATER AND WASTEWATER

March 6-12, 1974

ATTENDEES

AKAZAWA, Eugene T. Environmental Health Specialist	State Dept. of Health Lihue, HI
AKI, Paul F. Acting Chief, Pollution Investigation and Enforcement Branch	State Dept. of Health Honolulu, HI
ALLEN, William Environmental Engineer	R.M. Towill Corp. Honolulu, HI
ANAMIZU, Thomas M. Environmental Health Specialist	State Dept. of Health Honolulu, HI
BEENE, Janice C. Specialist	University of Hawaii Honolulu, HI
BONNET, William A. Project Engineer	Austin, Smith & Assoc., Inc. Honolulu, HI
CHANG, Bei-Jiann Graduate Assistant	University of Hawaii Honolulu, HI
CHASE, Robert Environmental Health Specialist	Dept. of Health Wailuku, Maui
CHEN, Ben Design Engineer	R.M. Towill Corp. Honolulu, HI
CHOW, Randy Chemist	State Dept. of Health Honolulu, HI

WORKSHOP ON
SAMPLING, MONITORING AND ANALYSIS OF WATER AND WASTEWATER

March 6-12, 1974

AGENDA

March 6, 1974	Wednesday
8:30	Registration
9:00	Introduction (DeFalco, Minette)
	Federal Requirements for Sampling, Sample Handling, Analyses, Quality Assurance, WQ Act (Shimmin)
10:00	Coffee Break
10:15	Survey Planning - Site Selection (Tunzi)
12:00	Lunch
1:30	Parameter Selection (Tunzi)
2:15	Coffee Break
2:30	Monitoring and Flow Measurement (Hathaway and Walz)
3:30	Compliance Monitoring (Wills)
3:45-4:45	Comparison of Sampling Methods in Waste Effluents (Kumagai)
March 7, 1974	Thursday
8:30	Monitoring and Flow Measurement (Hathaway and Walz)
10:00	Coffee Break
10:15	Instrumentation (Walz)
12:00	Lunch
1:30-5:00	Field Exercise (Johnson, Shimmin, Tunzi)
March 8, 1974	Friday
8:30	Discussion of Field Exercise -questions
9:00	Chemistry (Young) Federal Register-304 g Guidelines
10:15	Coffee Break
10:30	Bacteriology (Shimmin) Quality Assurance
12:00	Lunch

AGENDA - Continued

March 8, 1974	Friday (continued)
1:30	Statistics
2:30	Coffee Break
2:45-4:30	Biostimulation, Toxicity (Tunzi)
March 11, 1974	Monday (LABORATORY)
8:30	Registration (New Attendees)
9:00	Introduction, Federal Requirements (Shimmin)
9:30	Bacteriology (Shimmin, Johnson)
12:00	Lunch
1:30	Chemistry (Young) Demand, Nutrient, Oil and Grease
3:00	Coffee Break
3:15-5:00	Chemistry (Young) Demand, Nutrient, Oil and Grease
March 12, 1974	Tuesday
8:00	Heavy Metals (Young) Pesticides
10:00	Coffee Break
10:15	Bioassays (Tunzi)
12:00	Lunch
1:30	Biological Methods Manual (Tunzi)
2:30	Coffee Break
2:45-5:00	Quality Assurance (Shimmin)

ATTENDEES (Continued)

FUJIMOTO, Stanley Graduate Student	University of Hawaii Honolulu, HI
GEE, Henry K. Associate Res.	V.H. Water Resources Res.Ct Honolulu, HI
GLENN, Gail O. Biological Technician	U.S. Navy, Pacific Div. Navy Facilities Honolulu, HI
GOO, Reginald Chemist	State Dept. of Health Honolulu, HI
HARUNO, Jerry Environmental Health Specialist	State Dept. of Health Honolulu, HI
HASHIMOTO, William Y. Environmental Health Specialist	State Dept. of Health Kealekekua, HI
HAYASHI, Robert G. Water Microbiologist IV	Honolulu Board of Water Supply Honolulu, HI
HENDRICKS, Katherine L. Environmental Health Specialist II	State Dept. of Health Honolulu, HI
HIGUCHI, Gerald T. Environmental Health Specialist II	State Dept. of Health Honolulu, HI
HIRATA, Shiro Environmental Health Specialist	State Dept. of Health Honolulu, HI
ORI, Daniel Environmental Health Specialist III	State Dept. of Health Honolulu, HI

ATTENDEES (Continued)

HORIGAN, Laura L. Laboratory Director	R.M. Towill Corp. Honolulu, HI
ISHIKAWA, Gordon N. Environmental Scientist	Tripler Army Medical Ctr. Honolulu, HI
KANSAKO, Sidney I. Sanitary Chemist	City Hall, BWS (Sewers)
KARELITZ, Charles W. Chemist III	State Dept. of Health Honolulu, HI
KAWAMOTO, Ted Environmental Health Specialist	State Dept. of Health Honolulu, HI
KAWANISHI, Glenn Environmental Health Specialist	State Dept. of Health Hilo, HI
KILLIN, Robert D. Preventive Med NCO	Tripler Army Med Ctr Honolulu, HI
KONNO, Stanley Graduate Student	University of Hawaii Honolulu, HI
KROCK, Hans J. Sanitary Engineer	Sunn, Low, Tom & Hara, Inc. Honolulu, HI
KUBOTA, Edwin Y. Reg. Sanitarian IV	State Dept. of Health Honolulu, HI
KUMAGAI, James S.	Sunn, Low, Tom & Hara, Inc. Honolulu, HI
KUNIOKA, Robert T. Microbiologist	State Dept. of Health Honolulu, HI

ATTENDEES - (Continued)

KURAMOTO, Edward Environmental Health Specialist III	State Dept. of Health Honolulu, HI
LAFITAGA, Pasesa District Sanitarian	Environmental Health Branch Pago Pago, American Samoa
LEE, Bobby Analytical Chemist	Brewer Chemical Corp. Honolulu, HI
LEE, Daiwun Environmental Health Specialist III	State Dept. of Health Honolulu, HI
LEE, David Sanitary Chemist	Co. of Hawaii, Dept of Public Works Hilo, HI
LINDSAY, Stephen A. Biological Tech/Chemist	U.S. Navy Nav Fac. Eng. Cmd. Pearl Harbor, HI
LOW, Lionel Laboratory Director	Sunn, Low, Tom & Hara, Inc. Honolulu, HI
McCARTER, Mildred L. Chemist III	State Dept. of Health Honolulu, HI
MIYAMOTO, Howard Environmental Health Specialist I	State Dept. of Health Honolulu, HI
MONCRIEF, Robert Marine Biologist	Dept. of Defense (Navy) Pac. Div. NAFFACENGCOM Makalapa, HI
MURANAKA, Harry Sanitary Chemist	County of Kauai Lihue, Kauai

ATTENDEES - (Continued)

NAKANISHI, Ernest N. Sr. Chemist	Brewer Analytical Lab Honolulu, HI
NAKAMURA, Sidney S. Lab Assistant	BWS - Sewers City Hall
NAKASONE, Samuel S. Chemist	Board of Water Supply City Hall
NG, Earl W.M. Graduate Student	University of Hawaii Honolulu, HI
ONO, Wayne Chemist III	State Dept. of Health Honolulu, HI
OUMI, Charles Environmental Health Specialist	State Dept. of Health Honolulu. HI
POROTESANO, Ati Lab Technician	L.B.J. Medical Ctr. Pago Pago, American Samoa
PRIOR, Timothy Environmental Health Specialist	State Dept. of Health Honolulu, HI
RADCLIFF, Lester Microbiologist IV	State Dept. of Health Honolulu, HI
RICHARDSON, George C. Civil Engineer IV	City & County of Honolulu Board of Water Supply Div of Sewers Honolulu, HI
SHIGETANI, Mike Engineer	Board of Water Supply Honolulu, HI
SHIMABUKURO, Seichi Chemist	Federal Government, PWC Honolulu, HI

ATTENDEES - (Continued)

SHIRATORI, Bernard Environmental Health Specialist	State Dept. of Health Honolulu, HI
SHISHIDO, Kazuo Environmental Health Specialist	State Dept. of Health Honolulu, HI
SIMAO, Anthony M. Microbiologist	State Dept. of Health (Labs) Hilo, HI
SURFACE, Stephen W. Chemist	U.S. Navy Pac. Div., Nav. Fac. Eng. Honolulu, HI
TILLMAN, R. Bruce Environmental Health Specialist III	State Dept. of Health Honolulu, HI
TOKUNAGA, Edward H. Chemist	Board of Water Supply Honolulu, HI
TOMITA, Wayne Student	University of Hawaii Honolulu, HI
TSUTSUI, Roy Research Assistant	University of Hawaii Honolulu, HI
UYEMA, George M. Civil Engineer V	Board of Water Supply D/S C&C of Hon. Honolulu, HI
VICTOR, David Student	University of Hawaii Honolulu, HI
WAKATSUKI, Helen H. Chemist V	State Dept. of Health Honolulu, HI

ATTENDEES - (Continued)

WARD, Lawrence J. Preventive Med NCO	Tripler Army Med Ctr. Health & Env Service APO 96438
WILBERTS, Carson E. Environmental Health Specialist	State Dept. of Health Honolulu, HI
WONG, Darryl E. Grad. Assistant W.R.R.C.	University of Hawaii Honolulu, HI
YAMAMURA, Paul Microbiologist III	DOH Lab Branch W'Ku, Maui
YAMANE, George Environmental Health Specialist	State Dept. of Health Honolulu, HI
YIM, Spencer Graduate Student	University of Hawaii Honolulu, HI
YOSHINAGA, Glenn Student	University of Hawaii Honolulu, HI
YOSHINUTSU, Ushijima Sanitary Chemist	C&C BWS Sewers City Hall Honolulu, HI

PUBLICATIONS

The following is a list of publications disseminated to participants of the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater. These publications can be obtained through the Government Printing Office, or by contacting the appropriate EPA office, as indicated.

Abbreviated List of Publications and Guideline Documents Dealing with Monitoring Quality Assurance. EPA, Quality Assurance Division, Office of Monitoring Systems, Washington, D.C. 20460. January 1974.

Analytical Quality Control in Water and Wastewater Laboratories. EPA, National Environmental Research Center - Cincinnati, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268. (Technology Transfer, GPO-1972-479-971) June 1972.

Biological Field and Laboratory Methods; for measuring the quality of surface waters and effluents. EPA, National Environmental Research Center - Cincinnati, Office of Research and Development, Cincinnati, Ohio 45268, (EPA-670/4-73-001) July 1974.

Methods for Chemical Analysis of Water and Wastes. EPA, National Environmental Research Center - Cincinnati, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, (GPO-5501-0067) 1971.

Monitoring Industrial Wastewater. EPA, Office of Technology Transfer, Washington, D.C. 20460, August 1973.

Two "Technology Transfer" audio-visual instruction units were presented as part of the March 11, 1974 lecture on "Demand, Nutrient, and Oil and Grease."

1) "Determination of Grease and Oil"
   58 slides, 15 minute tape, script
   (# XT-56)

2) "Determination of Total Organic Carbon"
   59 slides, 13 minute tape, script
   (# XT-59)

Both instructional units are available on loan from either the EPA, National Training Center, Cincinnati Ohio 45268, or the EPA, Region IX, Air and Water Division, Manpower Training and Development, San Francisco California 94111.

FEDERAL REQUIREMENTS

FOR

SAMPLE HANDLING, ANALYSES, QUALITY ASSURANCE

By

Kathleen Shimmin
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

FEDERAL REQUIREMENTS FOR MONITORING

The following sections of PL92-500 apply to monitoring:

Sec. 106 - State Program Grants
Monitor water quality within State
Determine validity of data and update data annually
Specific requirements of Appendix A

Sec. 304 - Effluent Guidelines

304b - Identify constitutents and chemical, physical and biological character of the effluent

304g - Analyze according to methodology guidelines published in the Federal Register, 10/6/73

304h - Requires information on point source discharges including specific requirements on monitoring and reporting

Sec. 305 - Water Quality Inventory
Annual report on quality of all navigable water to be submitted by each State

Sec. 308 - Inspections, Monitoring, Entry

308a - Install, use, maintain monitoring equipment or methods according to EPA-prescribed guidelines
Sample according to method, location, and frequency required by EPA

308c - State should set up procedures for inspecting and monitoring point source discharges. These are to be submitted to EPA for approval.

Sec. 402 - National Pollutant Discharge Elimination System (NPDES) - Permits
After permit issued, inspection and monitoring is required of permittee - self-monitoring
Necessary for State to have system of judging

validity and adequacy of self-monitoring data reported. One way is to have periodic analyses of effluent by another laboratory.

California - writing into permit that data must be supplied by certified lab.

CHAIN-OF-CUSTODY PROCEDURES

These procedures have been adequate for EPA, Region IX, Surveillance and Analysis Division, Microbiology Section.

1) Take sample and write on label: sampler; witness; time; date; location or identifying location number.

2) Safeguard sample at all times, by either having it within view or locked up.

3) Sign when custody transferred, (custody tags have place for this).

4) Note in laboratory notebook: name of sampler; name of analyst; time of sampling and of beginning of analysis.

5) After bacteriological sample analysis has been initiated (i.e., sample has been inoculated into medium) paste label from sample bottle (label contains information listed in (1)) into laboratory notebook assigned to the particular field study.

6) Make a record of all observations in this notebook. Observations include colony counts, MPN codes, calculations, and final density determinations. Record also biochemical and serological reactions.

7) Include temperature records for waterbath in notebook.

8) Store notebook in locked cabinet.

9) If the sample were for chemical analyses and could be stored, then the sample too should be stored in a locked cabinet, together with the chain-of-custody tag, until the enforcement case is over.

Photographic Documentation

1) Labels showing sample station location and date can be included in photograph by having a sign included in the picture.

2) In notebook, record direction of camera.

3) If possible, include identifying landmarks in photo and then take a progressive series of closeups of problem.

4) Color film is recommended over black and white film, unless photo to be published in black and white.

COMPLIANCE MONITORING

By

Carroll G. Wills
EPA, NFIC-Denver
Denver CO

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

COMPLIANCE MONITORING

I. Introduction

Primary responsibility to prevent, reduce and eliminate water pollution rests with the States

Current EPA priorities: promulgate effluent guidelines and issue permits until States have authority (8 States have program)

~65,000 permit applications

EPA Goal: issue all major permits by December, 1974

II. Legal Authority

Sec. 106 - To obtain grants for administering pollution control programs, states must have program to monitor quality of navigable waters and groundwaters

Sec. 304(h) - State program must include monitoring; EPA promulgate guidelines for monitoring for state 402 programs

Sec. 402(a)(3) - EPA permit program subject to same terms and conditions as apply to state permit program

III. Major Objectives of EPA Compliance Monitoring Strategy

Document effectiveness of State agency monitoring and enforcement program

Document effectiveness of permittee's self-monitoring and reporting

Document violations of NPDES permit conditions and water quality standards

Provide evidentiary support to litigation

EPA strategy recognizes importance of permittee self-monitoring and reporting system for identifying compliance schedule and effluent limitation violations.

IV. Compliance Monitoring Program Elements

A. Facilities Inspections

B. Case Preparation Monitoring Investigations

C. Review of Self-Monitoring Reports

a) Schedules

b) Effluent Limits

D. Information System

E. Quality Assurance

F. Ocean Dumping (where applicable)

G. Non-filers and False or Fraudulent Information

H. Special Actions (Emergency Powers, Citizen Suits, Section 402(h) actions)

FLOW MEASUREMENT

By

James Hathaway and
and
Laurence Walz
EPA, NFIC-Denver
Denver CO

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

FLOW MEASUREMENT

Introduction

One of the most critical parameters in a compliance monitoring system is flow. Most current regulations and permits are written to allow either a net weight of pollutants (kg/day) or net weight of pollutants per process unit manufactured (kg/kkg of product) to be discharged. In order to be able to verify these regulations flow measurements taken must be as accurate and precise as possible. The installation of a flow device or calibration of an existing one will require a great deal of effort and ingenuity as well as planning to insure accuracy of the measurement.

If an existing flow device is available at the sampling point it is necessary to determine that the measurements are accurate. This may be done by checking the flow on an instantaneous basis. For instance, a flume or wier can be checked by measuring the head on the measuring structure and checking the flow tables to determine if the flow recorder is measuring correctly. Care should also be given to make sure that the structure is installed according to the appropriate design criteria, i.e., proper crest to head ratio, is structure leveled properly, is head measured in its proper place.

March 7, 1974

I. Open channel flows include sewers, open conduits, ditches, streams, and rivers. Flow measurements in open channels can be accomplished in the following way:

A. Instantaneous flows are measured with 1) velocity meters, 2) bucket and stop watch, or 3) calculated from flow equations.

1. Velocity meters are of three basic types:

a. The Price meter is the most commonly used for larger type flows, i.e., streams and rivers.

(1) The Price meter can be used on a wading rod (shallow water depths) or on a hand line when flows are measured from a bridge, suspension cable, boat, or other structure. A hand line is normally used when water depth or velocity prohibits wading.

(2) A rating table is normally furnished with meters when purchased. The curve should be checked to ascertain whether the rating was done for a hand line or rod suspension.

(3) Measurement is made by immersing the meter to a prescribed depth (0.6 of the depth for a d <2 ft, 0.2 and a reading at 0.8 for a >2 ft the average of the velocities is used) and counting the revolutions the wheel makes in a minimum of 40 seconds. The depth of the water and distance from shore are also measured. These data are recorded and used in calculating the flow.

Enough measurements should be made to completely define the stream cross section. If the cross-section is defined adequately, that is measuring all changes in the substrat, no more than 10 percent of the total flow will be measured in any one section.

b. Propeller type meters are often used in clean waters such as estuaries and bays. This type of meter should not be used where solids or sediments are present because of the clogging of exposed bearings.

c. Electromagnetic current meters work off of the principle of electromagnetic induction and therefore are not subject to clogging or interferences. The advantage of this meter is that they require very little area for measurement. This means measurements can be made very close to pipe walls without interference on velocity due to boundary conditions. Some of these meters will also measure both X and Y components of the current. These meters are direct reading and do not require calibration curves.

2. Discharge relationships can be established for sections of open channels if the section produces a large change in head for a small change in flow. The stage should be measured and measurements made of the stream flow at various stages. After enough data points have been collected, a rating curve can be established. Flow data can be interpolated from the curve but extrapolation of the curve may often cause the accuracy of the data to be questionable. The stage of the control section can be measured

by installing a staff gauge for instantaneous readings or recording continuous stages with a water level recorder.

3. Small flows (less than 30 gpm) discharging from pipes may be measured with a bucket and stopwatch. When this approach is used, a minimum of three repetitive measurements should be taken and averaged for the flow value.
4. If the resources are not available for flow measurement, in some instances the flow may be calculated. The following are methods generally used for such calculations:
   a. Horizontal or sloped open end pipe (Purdue method).
   b. California pipe.
   c. Manning equation.

   The accuracy of these methods is sometimes limited because of the inability to assign proper constants (friction constant and slope).

B. Continuous recording of flow conditions is desirable and should be done wherever possible. Various flow structures are available to give a reliable control which can be monitored continuously. Wiers and flumes are among these structures and are discussed below:

1. Wiers are most commonly installed for short-term monitoring because of the ease of construction and installation. Rectangular, Cipolletti and "V"-notch wiers can be constructed out of inexpensive plywood or light metal. If these wiers are properly installed, i.e., head conditions right and leveled, they will produce reliable and accurate flow measurements. When rags and other debris are present in the waste stream

clogging may occur at the wier crest and cause inaccuracies

The following flow ranges can be measured with these structures:

a. Rectangular wiers, .002 to 7,300 ft^3/sec.

b. "V"-notch wiers, .02 to 4 ft/sec^3.

2. Because of their self-cleaning properties, flumes are sometimes installed in conditions where clogging may occur. Flumes generally used are a) Parshall, and b) Palmer Bowles.

a. The Parshall flumes are often used in instances when continuous-flow measurements are needed for larger flows. The Parshall flume is sometimes more time consuming to set and seal in place, but proper selection of equipment can result in many years of trouble-free measurement. The Parshall must be leveled and all approach conditions met. Recording of the head is taken 2/3 of the distance on the converging section from the throat. If the downstream flow backs up and a ripple is evident downstream of the wier submergence is taking place. When this occurs it is necessary to measure the head on the throat as well as the upstream head. This measurement is necessary to determine the degree of submergence for use in the flow equation.

b. Palmer-Bowles flumes are easily installed and provide a cross-section which can be measured continuously. This type of flume can be blocked or sealed into a pipe or channel and will produce errors of less then 3 percent in the range of 10 to 90 percent pipe capacities. The

point of measurement is 1/2 the channel width upstream of the flume.

III. Flow in pressure conduits is more difficult to measure and requires more planning than that in open channels. Various meters are available, but because of expense of installation most are not conducive to a compliance monitoring program. Such meters include the following:

A. Venturi meters.

B. Pitot tube.

C. Magnetic meter.

D. Rotameter.

III. Miscellaneous Methods

A. Tracer dilution can be used for large flow systems but only provide an instantaneous measurement. The expense involved and man-hours required for this type of measurement is prohibitive in most cases. Some tracers commonly used are (1) lithium, (2) sodium chloride (3) flourescent dyes and (4) radioactive isotopes.

B. Tank volumes of batch discharges can be calculated by substraction of levels and then calculating the area of the structure involved.

C. Most facilities meter the incoming water rate for monthly billing. If the water use is known, an estimate can sometimes be derived by substracting product and process losses from this water use. At best this should be considered as only an estimate.

ORGANICS SAMPLER

AND

FIELD EXTRACTION PROCEDURE

By

Laurence Walz
EPA, NFIC-Denver
Denver CO

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

ORGANICS SAMPLER AND FIELD EXTRACTION PROCEDURE

The flow through the sampler is directed through a 3-way electric solenoid valve to the resin column, then through the metering system. The column consists of a stainless steel tube filled as follows:

a) 5 cm polyurethane foam plug

b) 50 grams of a 50-50 mixture of Amberlite XAD-2 and XAD-7 resins

c) 10 cm polyurethane foam plug

d) glass wool filter

Water for the liquid composite sample is taken off the system at the 3-way solenoid valve. The sample is pumped from the valve to a 19-liter sampling container which had been placed in a refrigerated cabinet. Extraction solvent (0.9 liters of freon or methylene chloride) is initially added to the sampling collection container. The solvent and sample are mixed continuously by means of a magnetic stirrer. The temperature of the refrigerated cabinet is maintained at 10°C to keep the extraction solvent from boiling off. Aliquots of sample (55 ml for a 72-hr composite and 80 ml for a 48-hr composite) are collected every 15 minutes. The time interval is controlled by a timer.

Extraction of the solvent from the composite water sample is made with a modified 1-liter Imhoff cone. The Imhoff cone should be enlarged to accommodate a 2-liter sample and a teflon stopcock attached to the bottom so that the extract can be drained off.

March 7, 1974

The sample extraction procedure used is as follows:

1. The Imhoff cone is cleaned by thoroughly rinsing with tap water followed by a careful rinse down the sides with 100 to 200 ml of acetone.

2. At the end of the sampling period, most of the water is discharged until only the solvent and about 500 to 700 ml of water are left. Measure and record the amount of water poured off.

3. Pour remaining solvent-water into the Imhoff cone and allow the two layers to separate for about one minute. Empty the organic layer into a sample container. Use 20 to 50 ml of solvent to clean the sides of the Imhoff cone. This solvent should also be decanted into the sample container.

4. Measure the amount of water left in the cone.

5. Add 50 grams of sodium sulfate to the solvent, carefully to avoid splashing. After the sodium sulfate is added, cap the container and shake vigorously for 30 seconds.

6. Record the date, sample location, and volume of water in the composite sample on the bottle containing the solvent.

7. Ice the sample for shipment to the NFIC-Denver laboratories for analysis.

Grab samples are collected in glass sample containers pre-rinsed in acetone and methylene chloride. Samples taken in the above containers are extracted with a freon or methylene chloride solvent.

A 2-liter glass separatory funnel is used as the separation container The sample extraction procedure used is as follows:

1. Separatory funnel is cleaned by adding 100 ml of solvent, mixed thoroughly and drained through the stopcock. This procedure is done twice.

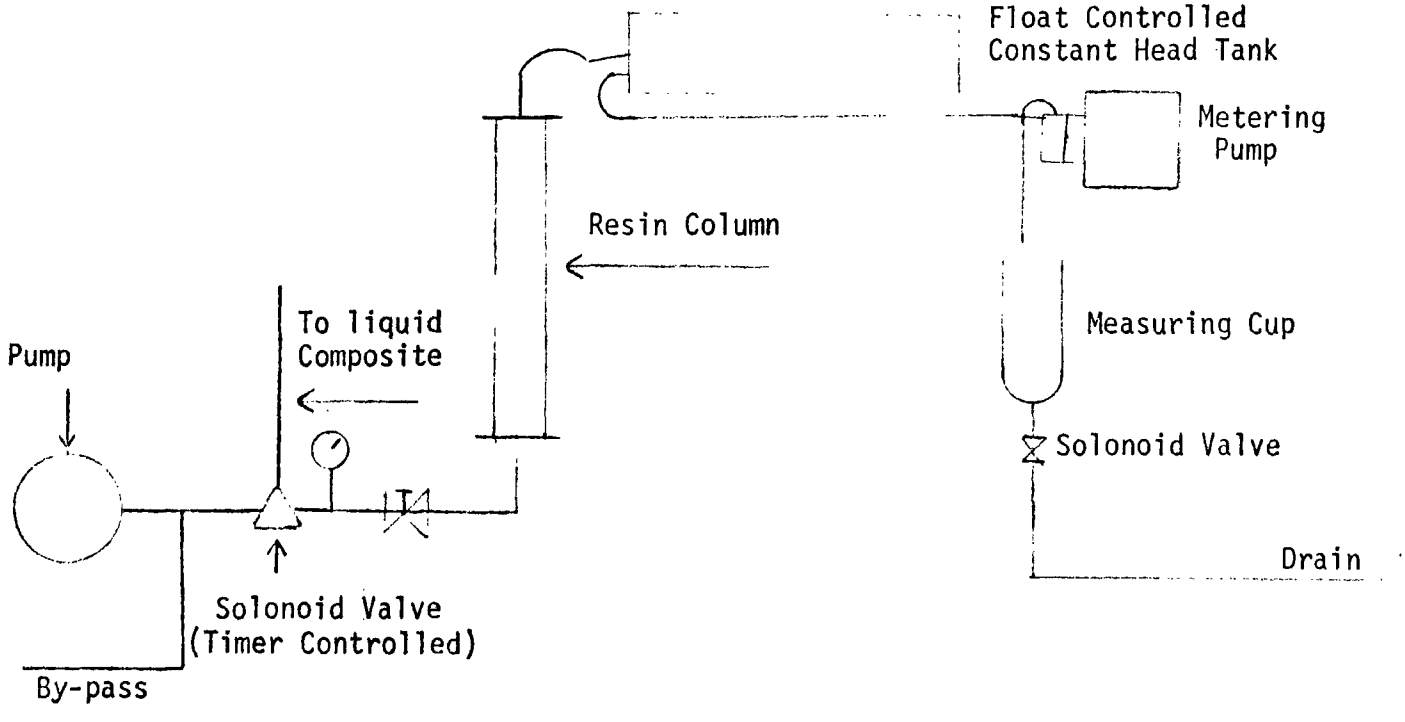
2. The water sample is poured from the sample bottle to the separatory funnel and 100 ml of solvent used to rinse the sample bottle.

3. The two layers are allowed to separate and the solvent layer drained into a 250 ml sample bottle.

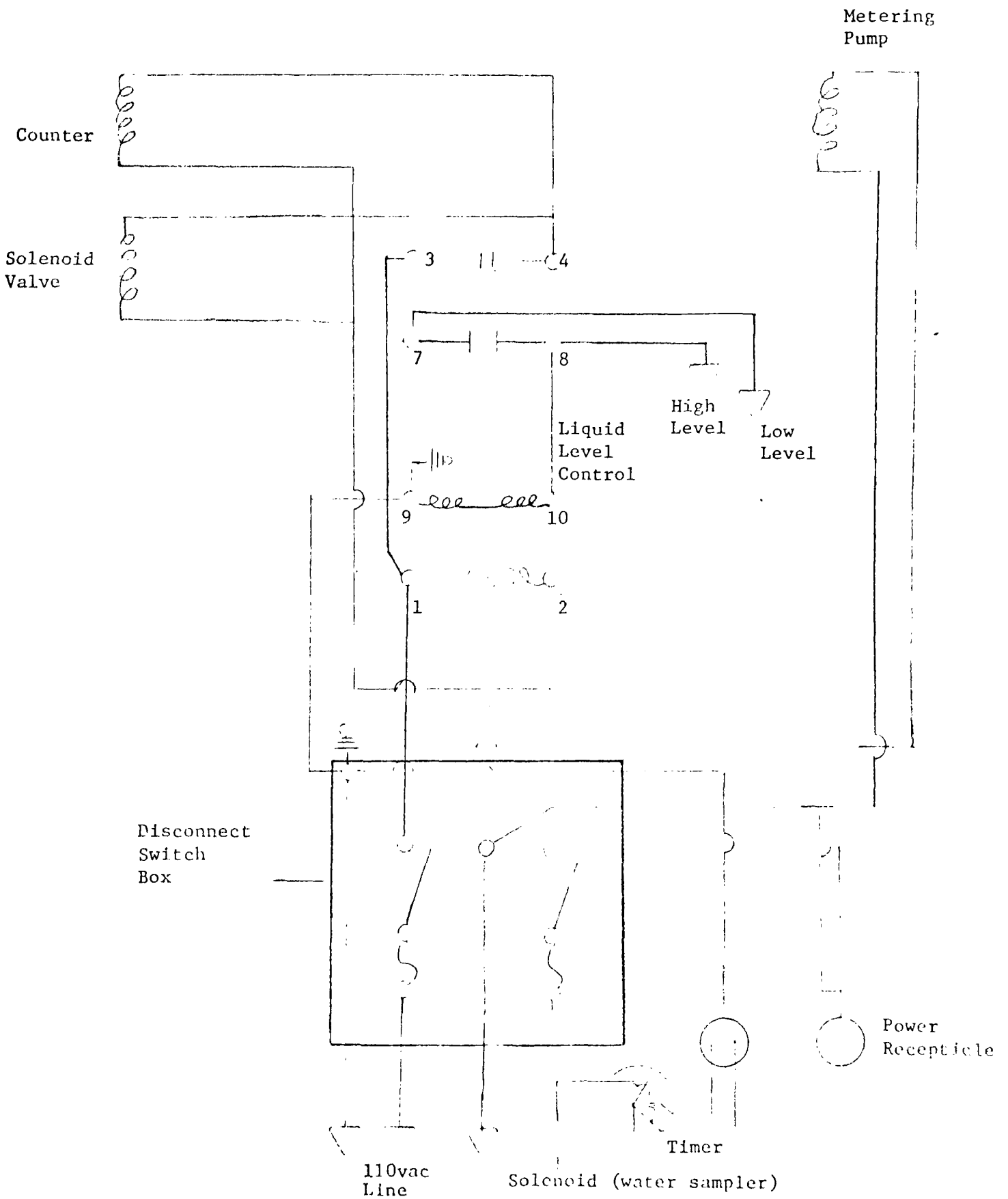
4. The extraction is repeated using 50 ml of solvent. When completed, this extract is added to the 250 ml sample bottle. Add 20 grams of sodium sulfate (20 grams) ice sample for shipment to NFIC-D laboratory for analysis.

SCHEMATIC, Organics Sampler

Flow Diagram



SCHEMATICS, Organics Sampler
Wiring Diagram
(Units With New Type Liquid Level Control)



COMPARATIVE SAMPLING RESULTS: SOME EXAMPLES

By

James S. Kumagai
Sunn, Low, Tom and Hara Inc.
Honolulu HI

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

COMPARATIVE SAMPLING RESULTS: SOME EXAMPLES

Some sampling results are presented here to illustrate the magnitude of water quality variations in wastewaters and in coastal waters. The results used here as examples were intended either for estimating the loads on wastewater treatment works or for evaluating water quality impact on coastal waters from wastewater discharges. The fact that water quality varies over a period of time requires a sampling program broad enough in scope to cover the spectrum of water quality values.

A. Program Variables

The variables considered here are two of the following:

1. Number of samples.
2. Frequency - how often and when.
3. Duration - over what period of time.

B. Constraints

1. Time
2. Budget
3. Maximizing information gained/unit effort

C. Guidelines for Sampling Program

1. Know purpose of sampling and what statistical parameters are most meaningful: maximum values, average, minimum, frequency distribution.
2. Work with cause-effect relationships: factors which affect water quality.
   a. wastewaters: production practices and rates; nature of raw materials
   b. receiving waters: surface and subsurface runoff, waste discharges; mixing, transport, reaction
3. Utilize available data on factors affecting water quality.
   a. stream flow data are available for Hawaii streams
   b. production rates are available for evaluating industrial discharges

c. certain seasonal oceanographic conditions are known from experience

4. Set up sampling frequency and duration to coincide with variations in the factors causing water quality changes (for example, seasonal changes).

5. Maximize information gained by correlating sampling results to causative factors or to quality indicators (for example, coral growth, fishes).

D. Examples

1. Municipal raw sewage sampling; required average values

Situation	Action
Limited Time & Budget	Three days, 24-hour composite
Limited Manpower	Single weekly grab samples over one year; 90 samples for Pearl City, 103 samples for Kailua

Result (Source: WQPO, 1971)

	Three Day Composite (ave mg/l ± std dev)	One Year Grab (ave mg/l ± std dev)
Pearl City STP		
BOD	233±85	267±88
Suspended Solids	183±13	282±125
Kailua STP		
BOD	138±51	80±40
Suspended Solids	120±34	151±63

2. Sugar mill washwaters; required: design suspended solids and impact on coastal waters (SLTH, 1972-1973)

Situation	Action
Limited Time	Composite samples, hourly samples selected over different seasons
Short Term/Long Term Variation Required	Correlation with cane harvesting and processing rates over 7-year periods; determination of concentration frequency curve for design

Results:

	Ton Soil/Ton Net Cane Mean and 95% Confidence Interval n=24, three days*
a. Season	
Dry	0.12±0.01
Rainy	0.19±0.04

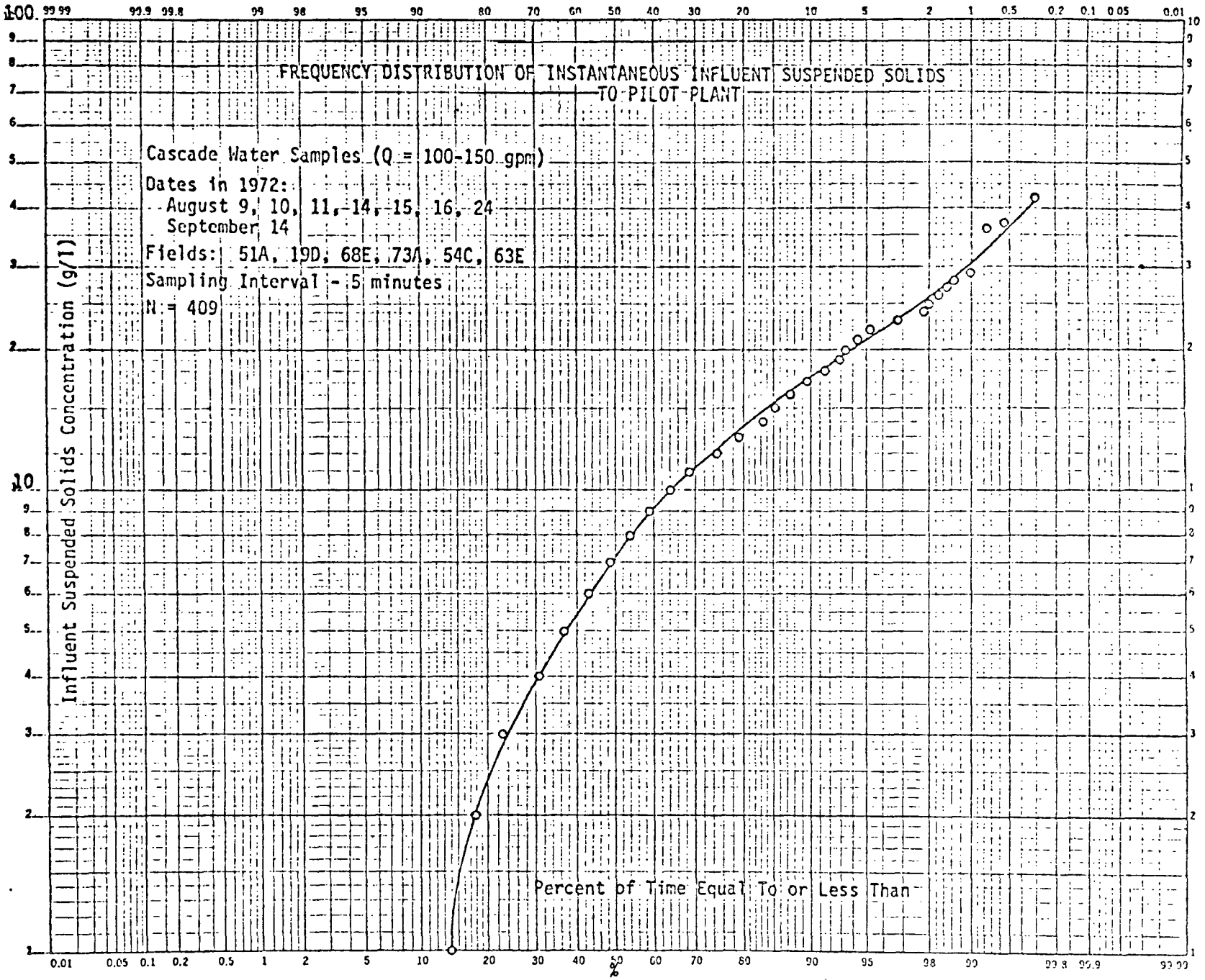
* These results were extended by correlating soil loads to trash in field cane to derive estimates of variations from day-to-day and year-to-year (see figure).

b. Frequency curve, see figure.

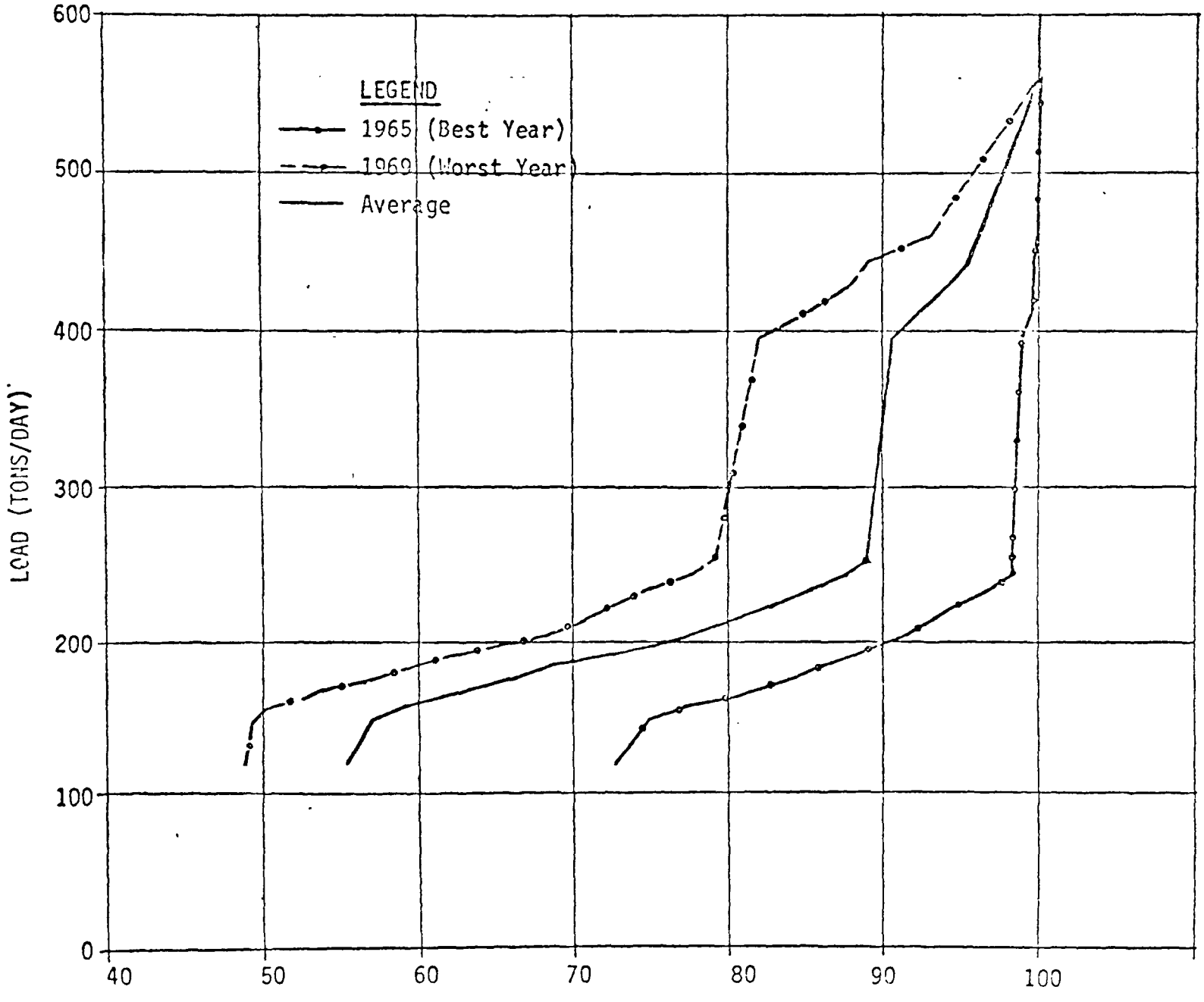
3. Coastal waters (SLTH, 1973)

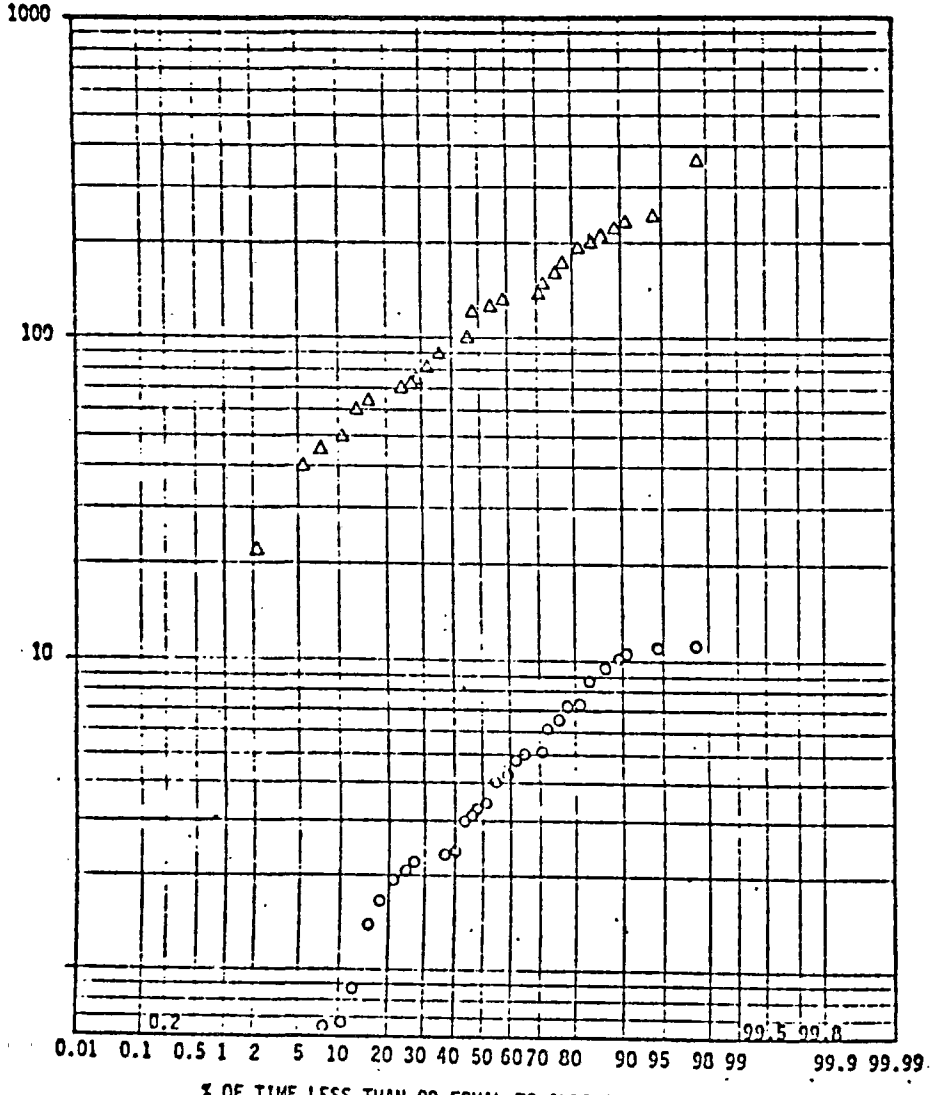
Situation	Action
Required: natural variation in water quality for comparison with sites close to sewage outfall	Monthly sampling over one-year concentration frequency curve

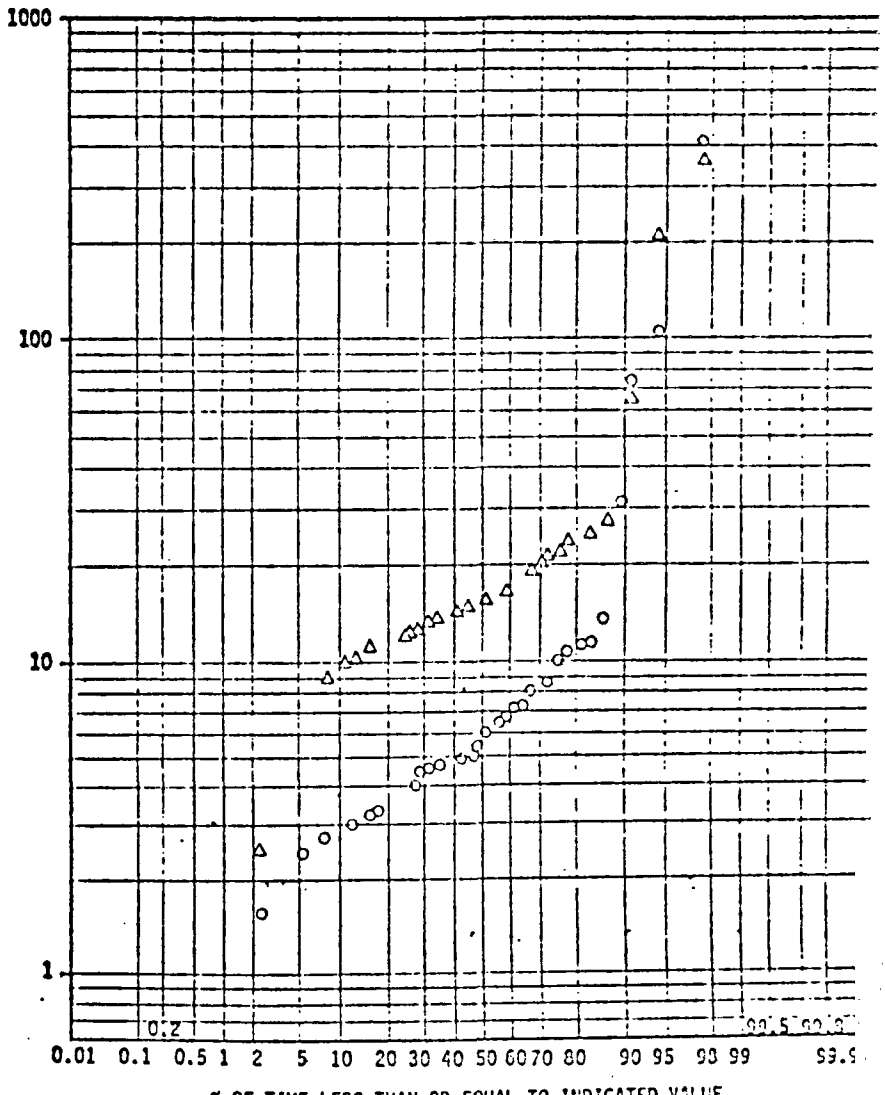
Result: (see figure)


FREQUENCY DISTRIBUTION OF INSTANTANEOUS INFLUENT SUSPENDED SOLIDS TO PILOT PLANT
Cascade Water Samples (Q = 100-150 gpm)
Dates in 1972:
August 9, 10, 11, 14, 15, 16, 24
September 14
Fields: 51A, 19D, 68E, 73A, 54C, 63E
Sampling Interval - 5 minutes
N = 409
Influent Suspended Solids Concentration (g/l)
Percent of Time Equal To or Less Than
%

CUMULATIVE FREQUENCY OF SOIL LOADS
TO WASTEWATER TREATMENT PLANT




1000
100
10
0.01 0.1 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99 99.9 99.99
% OF TIME LESS THAN OR EQUAL TO INDICATED VALUE
STATION 1
NO3-N, ug/l
TKN , ug/l


1000
100
10
1
0.01 0.1 0.5 1 2 5 10 20 30 40 50 60 70 80 90 95 98 99
% OF TIME LESS THAN OR EQUAL TO INDICATED VALUE
STATION 1
PO4-P, ug/l
TOT-P, ug/l

AN OUTLINE ON THE BACTERIOLOGY OF WATER

By

Kathleen Shimmin
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

BACTERIOLOGY OF WATER

Historically bacteria in water have been of concern as an indicators of potential disease transmission. Water-borne infections can be contracted through ingestion of or contact with contaminated water and also by ingestion of shellfish (filter feeders which concentrate particles in water). Some reports (Janssen and Meyers, 1968) have indicated that fish from contaminated waters may be affected by human pathogens with the concomitant possibility that the fish could be vectors of human disease.

I. Diseases Caused by Water-borne Pathogens

A. Typhoid fever - an infectious disease beginning with intestinal involvement and becoming systemic; fatality rate 2% with therapy and 10% in untreated cases; carrier state common.

1. Organisms - Salmonella typhi. Other Salmonella sp. and Shigella sp. may cause milder forms of gastroenteritis.

2. History

a) Budd - Essay on Typhoid Fever - 1856

b) Currently - still appears when water treatment systems break down

B. Cholera - an acute intestinal disease with rapid onset, profuse diarrhea and dehydration. Death rate may range from 5 - 75%.

1. Organism - Vibrio cholerae

2. History

a) Snow's study - Broad Street pump, 1854

b) Endemic in India - spreads from there

1830 - Pandemic in Europe
1817 - 1823; 1883 - 1896, U.S. epidemic
1826 - 1837 - Quebec

c) 1892 - Hamburg - Altona study
Hamburg got water from Elbe. Deathrate 134/100,000
Altona ran Elbe water through sand filter → Death rate 23/100,000.

Encouraged water treatment in Europe and England.

d) 1970 - Epidemic in Middle East - Turkey, Northern Africa (1000's of cases).

C. **Leptospirosis** - an acute infectious disease with fever and chills; jaundice may occur; fatality rate may approach 20%.

1. Organism - Leptospira sp. - enters through mucous membrane or break in skin.

2. Currently of importance in the U. S. and other parts of the world wherever people come into contact with contaminated waters; e.g., farm ponds and streams accessible to infected animals.

Many animals may carry organism - Rodents, cattle, horses, dogs, etc.

1970 - deaths of seals along California Coast- leptospirosis the cause.

3. Conditions favoring survival of leptospira - slowly flowing or stagnant water; pH slightly alkaline; temperature slightly high (22°C).

D. **Tularemia** - an infectious disease of animals and man; begins with chills and fever; usually an ulcer appears at site of original infection; fatality, 5% in untreated cases. Disease is spread by arthropod bite, by contact with infectious animals or by ingestion of contaminated food or water.

1. Organism - Franciscella tularensis (Pasteurella tularensis)

2. History

a) Epidemic in Russia 1937-43

Disease spread by water in wells and streams

b) Has been isolated from water in U.S. (including California) and other parts of world.

Avoid water-borne infection by not drinking untreated water in endemic areas.

E. Tuberculosis - a chronic well-known bacterial disease; usually pulmonary involvement, but systemic spread may also occur.

1. Organisms - Mycobacterium tuberculosis

Found in discharges from tuberculosis sanitoriums.
Conventional treatment removes part but not all t.b. organisms.
Chlorination is ncessary - can survive in water a long time.
Cases due to water-borne infection have been associated with near-drowning in contaminated water.

F. Viral diseases

1. Infectious hepatitis - most cases from direct person-person contact, but a low percentage are from water or from eating contaminated shellfish.

One of few viral diseases actually proven to be transmitted by contact with contaminated water.

A number of water-borne epidemics throughout the world
New Delhi in 1950's - over one million people were infected.

2. Poliomyelitis - little substantiated evidence of water-borne transmission.

3. Many potentially pathogenic viruses are found in feces and other discharges of man.

It is possible that studies may give more evidence for water-borne infections - those viruses found in human sewage include:

Enteroviruses: Coxsackie, poliomyelitis, ECHO (enterocytopathogenic human orphan)
Reoviruses
Adenoviruses
Rhinoviruses
Infectious hepatitis

G. Diseases can also be caused by protozoa and invertebrate animals in water.

Two among many examples:

1. Amoebic dysentery - caused by a pathogenic protozoan Endamoeba histolytica

2. Swimmer's itch - a dermatitis caused by swimming in waters contaminated by larvae of schistosomes of birds and rodents.

   People have been infected while bathing in lakes in the United States.

A more severe disease, schistosomiasis (blood fluke disease), which becomes a chronic infection involving the intestinal and urinary tracts, has not been found in the United States.

II. Bacterial Indicators of Pollution - General

A. Usually one can't test for pathogens directly.

1. Tests too time-consuming.

   Too difficult for routine work in a water laboratory.

   Negative recovery results give a false sense of security.

B. Characteristics of a good indicator organism

1. Applicable to all types of water.

2. Present when fecal contamination present and absent when fecal contamination not present.

3. Persists as long as the most resistant pathogen.

4. Can be easily and reliably identified in laboratory.

5. Not found as natural inhabitant of unpolluted environment.

C. Bacterial indicators are used in a variety of circumstances.

1. Tests for compliance with bacterial water quality standards.

   a) Drinking water, raw supply and finished water.

b) Water for specific purposes: shellfish cultivation, recreational waters to be used for primary and secondary body contact.

c) Enforcement of standards controlling waste discharge from industries and municipalities.

2. Treatment plant effectiveness evaluation.

3. Water quality surveys.

a) Detecting source and extent of pollution.

4. Special studies.

a) Epidemiological studies to detect source of pathogenic organisms

b) Investigations of problems caused by certain bacteria
Sphaerotilus, Clostridia

III. Coliforms - as Indicators

A. History and Description

1. In 1885 Escherich isolated from human feces an organism which he termed Bacterium coli-commune. The current name for the bacterium is Escherichia coli.

2. Escherich thought the bacterium to be peculiar to human feces. Since his time, however, Escherichia coli and other organisms from the coliform group have been recovered from polluted and unpolluted soils and from vegetation.

3. Standard Methods defines the coliform group as "gram-negative nonsporulating rods, which ferment lactose with the production of gas within 48 hours at 35°C".

4. The coliform group includes organisms from the following genera.

a) Escherichia
b) Aerobacter (Enterobacter)
c) Klebsiella
d) Erwinia
e) Serratia

B. General tests employed for detection of total coliform populations

1. Requirement and assumptions

Water quality standards may be written in terms of limiting numbers of total coliforms per given volume of water.

It is assumed that the bacteria present in a sample can be uniformly dispersed, so that accurate sample dilutions may be made. Either the sample or its dilution is inoculated into a given medium and incubated. The resulting growth allows one to assess the number of bacterial cells per volume in the original sample.

2. Tests

a) Multiple Dilution Broth Tube Method.

Replicate 10-fold dilution tubes are inoculated and incubated for 24-48 hours at 35°C.
(Lauryl Tryptose Broth for presumptive test, Brilliant Green Bile Lactose Broth for confirmed test).
A positive result is indicated by gas production. The numbers of positive tubes and their dilutions are noted. The Most Probable Number (MPN) of coliforms per 100 ml can be calculated by consulting tables in Standard Methods. The tables give estimates and confidence intervals for the original number of cells per volume in the sample. It should be emphasized that the MPN is a statistical estimate, not an actual count of the numbers of bacteria. Confirmed coliform results require a minimum of two days and a maximum of four.

b) Membrane Filter Method.

Various concentrations of a water sample are filtered through a membrane (cellulose acetate) filter. The filter is placed onto m-Endo medium and incubated for 24 hours at 35°C.

It is assumed that each bacterial cell deposited onto the filter (and capable of utilizing the medium employed - m-Endo, in this case for total coliforms) will replicate to form a colony.

The numbers of colonies present at given dilutions are noted.

The total number of coliform bacteria per 100 ml is calculated. This is an actual count, not a statistical estimate.

Time required for confirmed coliform results is 24 hours.

C. Differentiation of the coliforms: fecal vs non-fecal

1. Requirement

It is often necessary to distinguish between water contaminated with unpolluted soil and that contaminated with fecal material. A positive total coliform test does not make this distinction. Historically it was felt that Escherichia coli was characteristic of fecal contamination and Aerobacter aerogenes was typical of non-fecal contamination. (This is not necessarily true).

2. Tests

a) IMViC series. The letters are a mnemonic device to describe: Indole, Methyl Red, Voges-Proskauer, and Citrate.

i. Indole produced from metabolism of tryptophane, an amino acid. Reaction is typical for E. coli, not for A. aerogenes.

ii. Methyl Red test. When typical E. coli grow in glucose peptone broth, their fermentation brings the pH to 4.2 - 4.6 (methyl red indicator red, positive) and their growth terminates. The terminal pH for A. aerogenes in a similar culture medium is above 5.6 (methyl red indicator yellow, negative).

iii. Voges-Proskauer test. Typical A. aerogenes growing in glucose-peptone broth produce as a by product acetylmethyl carbinol (positive test); E. coli does not (negative test).

iv. Citrate test. Typical Aerogenes can utilize citrate as sole carbon source (positive test); E. coli cannot (negative test).

b) Elevated temperature tests. Underlying assumption for these tests is that organisms of fecal origin will grow at elevated temperatures (45°C), whereas those of non-fecal origin won't. Various media and conditions are employed. All procedures require incubation in a water bath (for accurate maintenance of temperature). Incubation should begin within 30 minutes of inoculation.

i. Eijkman test requires pure culture, lactose broth, 48 hours at 44.5± 0.2°C.

ii. EC Broth (the medium currently recommended), does not require pure cultures. Results are read after incubation for 24 hours at 44.5± 0.2°C. Geldreich (1966) states that the elevated temperature confirmatory test has an accuracy of correlation between positive coliform tubes and fecal origin (from warm-blooded animals) of 96%.

iii. Boric Acid Lactose Broth gives results similar to those from EC Broth. Incubation is 48 hours.

iv. $_{m}$FC medium may be used with membrane filters. Results are read after 24 hours incubation at 44.5± 0.2°C. According to Geldreich (1966) the accuracy of correlation between positive results by this method and actual fecal origin is 93%.

D. Applications

1. Total coliform test is used especially in evaluating potability of drinking water.

2. Bacterial standards have been established to determine acceptability of a water for a given use (eg., drinking water supply, recreation with primary or secondary contact, etc.). The standards are usually expressed in terms of total coliform counts and, with increasing frequency, may include also fecal coliform levels.

IV. Fecal Streptococci as Indicators

A. Background

Since coliforms can be found as natural inhabitants of unpolluted soil and since their die-off rate in seawater is rather rapid, there have been attempts to find additional indicator organisms to supplement the coliform tests. Fecal streptococci have been investigated in this regard since they are always present in the feces of warm-blooded animals, and since they are more persistent in seawater than are the coliforms. The ease with which the fecal streptococci could be detected and enumerated was increased by the use of azide dextrose broth, developed in 1950 (Mallmann and Seligmann, 1950).

B. Composition of the group

1. Standard Methods defines the fecal streptococci as the intestinal streptococci from all warm-blooded animal fecal wastes.

2. Fecal streptococci are gram-positive, spherical, chain-forming bacteria, which usually can develop at 45°C. Included are the following groups: enterococcus; S. mitis-salivarius; S. bovis; S. equinus; enterococcus biotype.

C. Relationships between fecal streptococci and fecal coliforms.

1. The fecal streptococci may be compared to fecal coliforms, since both originate from fecal sources. Ratios between the two groups may vary depending upon sources, methods of enumeration, and geographical location.

2. Generally, an FC/FS ratio of 2-4/1 indicates fecal pollution of human origin, whereas a ratio of less than 1/1 suggests fecal pollution of non-human, animal origin. The following table shows data, compiled by Geldreich (1966), describing the comparative densities of fecal streptococci and fecal coliforms in various warm-blooded animals, including man:

ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS

Animals	Avg wt of feces/24 hr wet wt, g	Average indicator density per gram of feces		Average contribution per capita per 24 hr		Ratio FC/FS
		Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.4

3. Identification of specific groups within the fecal streptococci may give an indication of certain characteristics of pollution: recentness and animal species involved. Organisms of the S. salivarius group are unique to humans (Kenner et al, 1960); S. bovis and S. equinus are usually not found in humans but predominate in cows, pigs, sheep, and horses; enterococcus and enterococcus biotype groups comprise the predominant flora of humans and fowl. Presence of S. salivarius indicates recent pollution, because the organism has a rapid die-off in surface waters.

C. Tests

1. Assumptions

As with the coliform tests, it is assumed that uniform dispersal of bacterial cells and that accurate dilutions are both possible.

2. Multiple Dilution Broth Tube Method

Replicate, 10-fold-dilution tubes are inoculated and incubated 24-48 hours at 35°C. (azide dextrose broth for presumptive test, ethyl violet azide broth for the confirmed test - both are listed in Standard Methods)

A positive result is indicated by turbidity of the broth.

By noting the numbers of positive tubes and their respective dilutions, one can calculate the MPN (see Standard Methods).

Confirmed results require four days of test time.

Recent reports (Buck, 1969) indicate that for marine waters the MPN method, using azide dextrose and ethyl violet azide broths, should include a final microscopic examination to insure that streptococci are indeed present in positive tubes. Nonstreptococcal growth has been observed in both these media following seawater inoculation.

The filters are placed onto KF medium (which has a higher recovery than m-Enterococcus medium) and are incubated for 48 hours at 35°C.

V. Other Bacterial Indicators of Pollution

A. Total bacterial counts

1. The term "total bacterial count" is fallacious

a) Methods which cultivate bacteria in the laboratory will recover only those bacteria which can grow in the growth conditions provided. It is not possible in the laboratory to provide all variations of environment simultaneously in the same growth medium.

b) Methods which enumerate bacteria directly (microscopic counts, turbidity measurements) do not distinguish between living and dead cells. Identity of the bacteria remains unknown.

2. General plate counts can be useful in detecting bacterial changes in a water source or treatment process. However, they give no indication of fecal origin of pollution and little identification of the bacterial species being cultured.

3. Historically plate counts were used to assess water quality.

   a) Robert Koch devised plate-count standards for safety of a water source (limit was 100 bacteria/ml, using gelatin medium, incubation 3 days at 20°C).

   b) Differential temperature counts have been used as indicators also. Duplicate plates were inoculated, one incubated at 20°C and the other at 37°C. When count ratios were compared, 20°/37° greater than 10 indicated non-polluted water; a ratio of one or less indicated polluted water.

B. Testing for miscellaneous indicators

1. Clostridium perfringens, a gram-positive, pathogenic, sporeforming rod, commonly found in soil and in feces of warm-blooded animals. Because the organism forms spores, it can exist in soil almost indefinitely. Its presence does not necessarily indicate presently-polluted water.

2. Pseudomonas aeruginosa, a gram-negative, pathogenic rod, which may be found in the intestinal tract of humans and warm-blooded animals. Since the organism is not found in large numbers in all humans, its value as an indicator is limited. (Sutter et al, 1967).

3. Viruses - may be assessed in the laboratory. However, special skills and equipment are required. Tests which can be carried out on a routine basis are still in the developmental stages.

C. Direct testing for pathogens in water is possible.

1. Applications

a) Direct testing is useful in tracing sources for epidemiological studies.

b) Direct study of pathogens is also required in special studies for it has been shown that pathogenic bacteria have been present in a water without this danger being reflected by routine coliform tests. (Greenberg and Ongerth, 1966; Seligmann and Reitler, 1965).

c) Used in shellfish studies (organism already concentrated by shellfish filtering system).

2. Limitations

a) A negative finding for a given pathogen does not mean that the water is safe from a public health standpoint.

b) Technical skills and equipment required to study pathogens are extensive and may not be found in every water laboratory.

c) Since the recovery of the pathogens (which are present, usually, at low levels) often depends upon concentration techniques, quantified results are not always obtainable.

VI. Considerations for Bacteriological Testing in Field Studies

A. Time of Processing

1. Collection and Preservation of Samples, General

According to recent work done in EPA laboratories in Cincinnati, Ohio and Edison, New Jersey, if there is any delay between collection and processing, the samples should be iced (but not frozen).

2. Fresh-water Samples

a) Properly iced samples should not be held for longer than four to six hours for <u>total coliform analysis</u>.

b) Properly iced samples for <u>fecal coliform analysis</u> should be processed within two to four hours.

3. Ocean-water Samples

a) Samples should be run within one hour. The maximum holding time (for properly-iced samples) is two hours.

b) Fecal coliform samples should be processed within 1/2 hour. A fecal coliform sample preserved for two to four hours is useful only for determination of general range of numbers.

B. Temperature and Time

1. The definition of the organisms recovered is based partially upon the temperature at which they grow. Therefore, it is critical that the incubation temperature be maintained strictly within the designated limits. Time allowed for growth is part of the definition also.

a) Total coliforms in multiple tubes, 48 hrs ± 3 hrs at 35° ± 0.5°C.

Total coliforms by membrane filter, 22-24 hrs at 35° ± 0.5°C.

b) Fecal coliforms in multiple tubes, EC medium, 44.5° ± 0.2°C for 24 hrs.

Fecal coliforms by membrane filter, mFC medium, 44.5° ± 0.2°C for 24 hrs.

Both these incubations should be in a water bath. Plates containing membrane filters are sealed in plastic bags (eg. Whirl-pak) to block water access.

c) Fecal streptococci by multiple tube and by membrane filter, 35° ± 0.5°C, 48 hrs.

C. Membrane-Filter Processing vs Multiple-Tube Technique (Coliforms)

1. Preparation time

a) Multiple-tube media for total and fecal coliforms may be prepared in advance.

b) Membrane-filter medium (mEndo) for total coliforms should not be prepared more than 72 hours

in advance. Medium for fecal coliforms (mFC) may be stored for 5 to 7 days.

2. Membrane-filter technique

   a) Advantages: confirmed results within 24 hours; test may be done entirely in field (media is not autoclaved); permanent record of results; less space necessary than for tubes.

   b) Limitations: suitable filtration volume must be selected; high turbidity interferes; large numbers of non-coliform inhibit appearance of coliforms; colonies must be recognized and counted; some percentage of counts should be verified by tube testing.

3. Multiple-tube technique

   a) Advantages: media may (must) be prepared in advance; positive test is easy to read; less interference from turbidity and large numbers of non-coliforms (than is the case with membrane filter technique).

   b) Limitations: media cannot be prepared in field; confirmed results require a minimum of 48 hours and a maximum of 96 hours incubation; a large amount of storage and incubator space is required (as compared to that necessary for membrane filters).

MOST PROBABLE NUMBER CALCULATION

Replicate ten-fold dilutions of the sample are inoculated into the appropriate broth and incubated. Following incubation, the numbers of positive tubes at each dilution are noted in order. The 3-number code which is formed may be looked up in MPN tables for an estimate of the number of bacteria per 100 ml.

The tables are based upon inocula of 10 ml, 1 ml, 0.1 ml sample. If the concentrations you use are different from these, multiplication by the appropriate power of 10 is necessary to put your results in the correct range.

Examples (5-tube tests)

	Results				Code	95% Confidence Limits		MPN/100 ml
						Lower	Upper	
a.	+++++ 10 ml	++--- 1.0 ml	+---- 0.1 ml		521	23	170	70
b.	+++++ 10 ml	+++++ 1 ml	++--- 0.1 ml	++--- 0.01 ml	522	280	2200	940
c.	----- 10 ml	+---- 1 ml	----- 0.1 ml		010	<.5	7	2
d.	----- 10 ml	----- 1 ml	----- 0.1 ml		000			<.2
e.	+++++ 10 ml	+++++ 1 ml	+++++ 0.1 ml		555			>2400

GRAM'S STAIN

1. Using a clean slide, make a thin smear of the culture on the slide.

2. Air dry

3. Heat fix by passing slide briefly through flame.

4. Add gentian violet dye - let stand one minute. Rinse with tap water. Rinse with Gram's iodine.

5. Add Gram's iodine - let stand one minute. Drain slide.

6. Add decolorizer - let stand 10-15 seconds. Rinse with water. Rinse with safranin.

7. Add safranin dye to counterstain - one minute. Rinse with tap water. Blot dry with paper towel.

   View slide under microscope.

   Gram-positive organisms appear blue-violet; gram-negative bacteria retain only the counterstain and hence appear red when safranin is used.

REFERENCES

Control of Communicable Diseases in Man, 10th ed., APHA. J. E. Gordon, Ed. Published by American Public Health Association, 1970 Broadway. New York, N.Y. 1965.

Standard Methods for the Examination of Water and Wastewater, 12th ed., APHA, AWWA, WPCF. Published by American Public Health Association, 1790 Broadway, New York, N.Y. 1965.

Buck, J. D. Occurrence of False-Positive Most Probable Number Tests for Fecal Streptococci in Marine Waters. Appl. Microbiology. 18:562. 1969.

Cockburn, T. A. and Cassanos, J. G. Epidemiology of Endemic Cholera. Public Health Reports. 75:791. 1960.

Diesch, S. L. And McCulloch, W. F. Isolation of Pathogenic Leptospires from Waters used for Recreation. Public Health Reports 81:299. 1966.

Geldreich, E. E. Sanitary Significance of Fecal Coliforms in the Environment. U. S. Department of the Interior. FWPCA Publ. WP-20-3. 1966.

Greenberg, A. E. and Kupka, E. Tuberculosis Transmission by Waste Waters - A Review. Sewage and Industrial Wastes 29:524. 1957.

Greenberg, A. E. and Ongerth, H. J. Salmonellosis in Riverside, California. Journal AWWA. 58:1145. 1966.

Janssen, W. A. and Meyers, C. D. Fish: Serologic Evidence of Infection with Human Pathogens. Science. 159:547. 1968.

Jeter, H. L. Bacteriological Indicators of Water Pollution, in Water Quality Studies, U. S. Department of the Interior, FWPCA Training Program. October 1969.

Kabler, P. W. et al. Public Health Hazards of Microbial Pollution of Water. Proceedings of the Rudolfs Research Conference. 1961.

Kenner, B. A. et al. Fecal Streptococci II. Quantification of Streptococci in Feces. Am. J. Public Health. 50:1553. 1960.

Mallman, W. L. and Seligman, E. B., Jr. A Comparative Study of Media for Detection of Streptococci in Water and Sewage. Am. J. Public Health. 40:286. 1950.

Metcalf, T. G. and Stiles, W. C. Enteroviruses Within an Estuarine Environment. Am. J. Epidemiology. 88:379. 1968.

Prescott, S. C., et al. Water Bacteriology. John Wiley and Sons, Inc. 1946.

Seligmann, R. and Reitler, R. Enteropathogens in Water with Low Esch. coli Titer. Journal AWWA. 57:1572. 1 65.

Sutter, V. L., Hurst, V., and Lane, C. W. Quantification of Pseudomonas aeruginosa in Feces of Healthy Human Adults. Health Laboratory Science. 4:245. 1967.

Taylor, F. B. et al. The Case for Water-borne Infectious Hepatitis. Am. J. Public Health. 56:2093. 1966.

This outline was prepared by K. G. Shimmin, Section Chief, Microbiology, Environmental Protection Agency, Region IX, 620 Central Avenue, Alameda, California 94501.

ANALYTICAL METHODS

FOR

METAL AND PESTICIDE ANALYSIS

By

Ho Young
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

ANALYTICAL METHODS FOR METALS

Metals that are analyzed in wastes consist of large numbers of cations of the alkali, alkaline earth, noble, heavy metal series etc.

I. Methods for metal analyses: The metal analyses depends on the structures of the metal ions, the energy levels of their electrons and the excitation energy.

   A. Emission flame photometry: It measures the amount of light emitted by the excited atom which is aspirated into a flame and atomized. It is commonly used for analyzing Na, K, Ca etc.

   B. Atomic absorption spectrophotmetry: This technique is different from the above in that this method measures the light absorbed. When a sample is aspirated into a flame and atomized, it absorbs at a certain wavelength of the light source.

      1. Instrument: Atomic absorption spectrophotometer.

         a. A light beam is directed through the flame into a monochromator, and onto a detector that measures the amount of light absorbed.

         b. Light absorption is more sensitive than light emission because it depends upon the presence of free unexcited atoms.

         c. In the usual flames, the ratio of the unexcited to excited atoms at a given moment is very high.

         d. Because each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed, making the method relatively free of spectral or radiation interferences.

         e. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

         f. Interference

            1. The most troublesome interference results from the lack of absorption of atoms bound in molecular combination in the flame which is not sufficiently hot to dissociate the new molecule.

ANALYTICAL METHODS FOR METALS

Metals that are analyzed in wastes consist of large numbers of cations of the alkali, alkaline earth, noble, heavy metal series etc.

I. Methods for metal analyses: The metal analyses depends on the structures of the metal ions, the energy levels of their electrons and the excitation energy.

A. Emission flame photometry: It measures the amount of light emitted by the excited atom which is aspirated into a flame and atomized. It is commonly used for analyzing Na, K, Ca etc.

B. Atomic absorption spectrophotmetry: This technique is different from the above in that this method measures the light absorbed. When a sample is aspirated into a flame and atomized, it absorbs at a certain wavelength of the light source.

1. Instrument: Atomic absorption spectrophotometer.

a. A light beam is directed through the flame into a monochromator, and onto a detector that measures the amount of light absorbed.

b. Light absorption is more sensitive than light emission because it depends upon the presence of free unexcited atoms.

c. In the usual flames, the ratio of the unexcited to excited atoms at a given moment is very high.

d. Because each metallic element has its own characteristic absorption wavelength, a source lamp composed of that element is employed, making the method relatively free of spectral or radiation interferences.

e. The amount absorbed in the flame is proportional to the concentration of the element in the sample.

f. Interference

1. The most troublesome interference results from the lack of absorption of atoms bound in molecular combination in the flame which is not sufficiently hot to dissociate the new molecule.

2. The presence of other atoms which absorb at the same wavelength.

3. Interference caused by ionization: Barium may undergo ionization in the flame and the ground state population is thereby reduced. This interference can be overcome by the addition of an excess of a cation having a similar or lower ionization potential.

2. Sample Preparation

a. Special Extraction Procedure: When the concentration of the metal is not sufficiently high to determine directly certain metals may be chelated and extracted with organic solvents.

1. Chelating agent: Ammonium pyrrolidine dithiocarbanate (APDC) for cadmium, iron, manganese, copper, silver, lead and hexavalent chromium.

2. Organic solvent: methyl isobutyl ketone (MIBK).

b. Digestion of sediment

1. Place 2 g of sediment in 250 ml beaker.

2. Add 10 ml of conc. HNO_3
0.5 ml of H_2O_2 (30%)

3. Cover beaker with a watch glass and allow mixture to gently reflux for 2 hrs. on a hot plate.[1]

4. Remove watch glass and evaporate to dryness. If the dry residue is a dark color, add a couple of drops of conc. HNO_3 and continue to heat. If the residue is still a dark color after repeating this process, proceed with the ashing.[1]

5. Ash the sample at 400-425°C for 1 hr. in a muffle furnace.

6. Cool to room temperature.

---

1 Modification by Alameda Laboratory, EPA, Region IX.

7. Add 10 ml of acid mixture[2].
   8 ml of 10% NH_4Cl
   0.4 ml of $Ca(NO_3)_2$[3]

8. Heat gently for 15 minutes and cool for 5 minutes or longer.

9. Transfer sample to a centrifuge tube - rinse the digestion beaker with 10 ml redistilled water until a final volume of 30 ml.

10. Centrifuge for 10 minutes at 20,000 RPM.

11. Transfer the supernatant into a 100 ml volumetric flask.

12. Rinse the digestion beaker with 30 ml of redistilled water and transfer into the centrifuge tube. Centrifuge and add the supernatant to the previous mixture.

13. Rinse and centrifuge a third and final time.

14. Adjust the final volume to 100 ml with redistilled water. The mixture is ready for AA analysis.

3. Fuel and oxidant combinations and wavelength settings needed for metal determinations, listed in the table below.

Metal	Fuel and Oxidant Combination	Wave Length nm	Sensitivity ug/l*	Interferences
Aluminum	Nitrous oxide-acetylene	309.3	1,000	Fe, HCl, V H_2SO_4 Ti acetic acid
Barium	" " "	553.6	200	
Beryllium	" " "	234.8	100	Al, Si, Mg
Cadmium	Air-acetylene	228.8	40	
Chromium	" "	357.9	150	Fe, Ni
Copper	" "	324.7	200	
Iron	" "	248.3	300	HNO_3 & Ni
Lead	" "	283.3	500	
Magnesium	" "	285.2	15	Si, Al, Na K, Ca
Manganese	" "	279.4	150	
Silver	" "	328.1	100	
Zinc	" "	213.8	40	Si

*ug/l of metal for 1% absorption in an aqueous solution.

---

2 200 ml conc. HNO_3, 50 ml conc. HCl, 750 ml redistilled H_2O
3 $Ca(NO_3)_2 \cdot 4 H_2O$ 11.8 g/100 ml

4. Analysis of Mercury: Flameless AA procedures

a. Procedure

1. The mercury is reduced to the elemental state and aerated from solution in a closed system.

2. The mercury vapor passes through a cell positioned in the light path of atomic absorption spectrophotometer

3. Absorbance, at 253 nm is measured as a function of mercury concentration.

b. Interference

1. Possible interference from sulfide is eliminated by the addition of potassium permanganate.

2. Copper: $>$ 10 mg/l

3. Chloride: During the oxidation step chlorides are converted to free chlorine which will also absorb radiation at 253 nm.

Prepared by
Ho Lee Young, Ph.D.
Chief, Chemistry Section
Laboratory Support Branch
EPA, Region IX

TABLE 1

Concentration Ranges

Metal	Detection Limit mg/l	Sensitivity mg/l	Optimum Concentration Range mg/l		
Aluminum	0.1	0.4	10	-	1000
Arsenic	0.05	1.0	10	-	100
Cadmium	0.001	0.004	0.1	-	2
Calcium	0.003	0.07	1	-	200
Chromium	0.01	0.02	1	-	200
Copper	0.005	0.04	0.1	-	10
Iron	0.004	0.006	0.1	-	20
Lead	0.01	0.06	1	-	10
Magnesium	0.0005	0.005	0.01	-	2
Manganese	0.005	0.04	0.1	-	20
Potassium	0.005	0.01	0.01	-	2
Silver	0.01	0.05	0.1	-	20
Sodium	0.001	0.003	1	-	200
Zinc	0.005	0.02	0.1	-	2

PESTICIDE ANALYSIS

Most of the pesticides used are synthetic, organic compounds. They can be grouped into organohalogens, organophosphates, organosulfates, organonitrogens and carbamates pesticides.

I. Analyses of Water and Wastewater Sample.

A. Extraction

1. One liter sample is extracted with 60 ml of 15% ethyl ether in hexane by shaking vigorously for 2 minutes.

2. Repeat extraction

3. The sample container is rinsed with each aliquot of extracting solvent prior to extraction of the sample.

4. Combine the organic solvents from both extractions.

B. Florisil clean up

1. Prepare a column by placing cotton plug in bottom, pouring 4" Florisil in column followed by 3/4" anhydrous sodium sulfate.

2. Pre-rinse column with 100 ml hexane and discard hexane.

3. Add extract to column (25-50 ml) and rinse beaker three times each with 5 ml hexane. Use caution in not allowing column to run dry.

4. When extract meniscus coincides with top of column packing, rinse column with 5 ml hexane; when meniscus again coincides with top of column packing, add 200 ml 10% ethyl ether in hexane to elute the sample.

C. Concentration of extracts

1. Collect the eluant in a 500 ml Kuderna Danish flask, fitted with an ampoule. Connect Snyder column to the flask and place in steam bath and evaporate until there is action in only two balls.

2. Remove Kuderna Danish flask from steam bath, tilt slightly and rotate to insure rinsing of all inner surface.

3. Concentrate the sample to 0.5 ml then remove the ampoule from the Kuderna Danish flask.

4. Analyze the sample using a gas chromatograph, without further dilution or concentration, unless the chromatogram indicates otherwise.

D. Gas chromatographic analysis

1. Instrument

a. Column: either spiral or U shape

1. Solid phase: Gas-chrom Q, 80-100 mesh, or 60-80 mesh.

2. Liquid phase: OV-17, OV-101, OV-210, QF-1

b. Carrier gas: Nitrogen, helium or argon.

c. Detector: Flame ionization and flame photometric detector, electron capture, coulometric detector, microcoulson electroconductivity detector.

d. recorder.

2. Temperature

a. Injection - port temperature: 220-230° C.

b. Column temperature: 175-200°C.

c. Detector temperature: Should be about 5-10° above column temperature.

3. Identification: Comparing with the retention time of the standards.

4. Quantitation: Computed from the area under the peak in the chromatogram.

II. Extraction of Pesticides from Sediments and Tissues

A. Extract

1. Mix the sediment thoroughly, measure up to 100 g into blender cup.

2. Add 100 ml acetonitrile and 60 g prehexane-rinsed anhydrous sodium sulfate and blend at moderate to high speed for 2 minutes.

3. Pour into (hexane rinsed) Buchner funnel and extract solvent.

4. Carefully transfer residue from Buchner funnel and filter paper into blender cup. Add another 100 ml mixed solvent, blend for 1 minute.

5. Pour into same Buchner funnel and filter paper. Extract solvent, combining extract with that obtained in step No. 3.

B. Back Partition

1. Transfer extracts to 1000 ml separatory funnel containing 400 ml distilled water, 100 ml sodium sulfate satuarated water, and 150 ml 5% ethyl-ether in hexane.

2. Shake vigorously 2 minutes, then let stand for 10 minutes, allowing the two phases to separate.

3. Save organic layer, washing twice with 50 ml portions of water.

4. Transfer to 250 ml beaker and evaporate over 70° C water bath to 25 ml.

C. Florisil clean up: Same as above

D. Concentration of extracts: Same as above

E. GC Analysis: Same as above

III. Thin layer chromatography for pesticides analysis

A. Extraction, clean up and concentration as above

B. The final solution is spotted on a glass plate covered with 0.25mm silica-gel G or magnesium oxide and developed in a solvent saturated chamber (hexane: acetone = 2:1)

C. If necessary, the developed spots can be made visible by spraying with an appropriate reagent, e.g. 1-naphthol.

D. Another identification technique is to remove the spot, extract the material, and run an IR scan.

Prepared by
Ho Lee Young, Ph.D.
Chief, Chemistry Section
Laboratory Support Branch
EPA, Region IX

Table 1
Summary of Gas Chromatographic Analyses of Pesticide Residues

	Extraction Solvent	Clean Up	Liquid Phase	Carrier Gas	Column Temp.	Detector
Carbamates	Methylene Chloride	Acetonitrile Partition Florisil Column				
Organo-halogens	15% Methylene Chloride in Hexane	Acetonitrile Partition Florisil Column	6% QF-1 + 4% SE-30	~~Argon~~ Methane	200° C	Electron Capture
Organo-nitrogen	Methylene Chloride	Florisil Column	1% Carbowax 20M	He	155° C	Electrolytic Conductivity
Organo-phosphate	15% Methylene Chloride in Hexane	Acetonitrite Partition Florisil Column	1.5% OV-17 + 1.95% QF-1	N_2	215° C	Flame Photometric Detector
Chlorinated phenoxy acid	ethyl ether		1.5% OV-17 + 2.95% QF-1 or 5% OV-210	~~Argon~~ Methane		Microcoulo-metric or Electrolytic Conductivity *or* Electron Capture

TABLE 2

RETENTION TIMES OF ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	3% DC-200 + 5% QF-1	5% OV-17	3% OV-101	3% OV-210	Relative Sensitivity to EC Detecto[2]
Column Temp.	200 C	200 C	175 C	160 C	
Pesticide	RRt[3]	RRt[3]	RRt[3]	RRt[3]	
α-BHC	0.40	0.45	0.33	0.54	1.0
Lindane	0.51	0.61	0.42	0.75	1.0
Heptachlor	0.80	0.79	0.76	0.82	1.0
Aldrin	1.00	1.00	1.00	1.00	1.0
Kelthane	1.19	1.52	1.12	2.46	0.1
Heptachlor Epoxide	1.38	1.58	1.30	2.16	0.5
γ-Chlordane	1.53	1.82	1.55	2.12	0.5
Endosulfan I	1.77	2.00	1.70	2.89	0.4
p,p'-DDE	1.93	2.67	2.18	2.91	0.5
Dieldrin	2.10	2.54	2.08	3.65	0.5
Endrin	2.43	3.21	2.33	4.46	0.3
o,p'-DDT	2.62	3.97	3.02	4.04	0.1
Endosulfan II	2.62	3.97	2.45	5.96	0.3
p,p'-DDD	2.68	4.13	2.94	5.61	0.1
p,p'-DDT	3.41	5.19	3.97	6.28	0.2
Methoxychlor	5.26	11.17	6.88	13.52	0.1
Aldrin (Minutes Absolute)	3.76	3.84	2.64	2.28	

[1] All columns glass, 6 ft. long x 4 mm ID, solid support Gas-Chrom Q (80/100 mesh), nitrogen carrier flow 80 ml/min.

[2] Sensitivity factors relative to aldrin.

[3] Retention times relative to aldrin.

304 (g) WATER QUALITY GUIDELINES

By

Ho Young
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

304(g) WATER QUALITY GUIDELINES

I. Section 304(g) of the Water Act requires that the Administrator shall promulgate guidelines establishing test procedures for the analysis of pollutants.

II. Guidelines were issued in the Federal Register, Vol. 38, No. 199, Part II, on October 16, 1973.

III. Objectives of guidelines establishing test procedures:

A. To establish reliable procedure(s) for analyses of various pollutants.

B. To assure the effluent discharge of pollutants from a point source or group of point sources meets the effluent discharge limitations set forth by Section 302.

C. To achieve the water quality in a specific portion of the navigable water which shall assure protection of public water supplies, agricultural, and industrial uses, protection and propagation of a balanced population of shellfish, fish and wildlife, and allow recreational activities in and on the water.

IV. Test procedure to be used by:

A. Any applicant for a Federal license or permit to conduct any activity including, but not limited to, the construction or operation of facilities which may result in any discharge into the navigable waters.

B. Permit applicants to demonstrate that effluent discharges meet applicable pollutant discharge limitations: National Pollutants Discharge Elimination System (NPDES).

C. The State and other enforcement activities in routine or random monitoring of effluents to verify effectiveness of pollution control measures.

V. Approved test procedures for pollutants and parameters:

Parameters	Units	Approved Test Procedures: Standard Methods[1]	ASTM[2]	EPA Methods[3]
General Analytical Methods				
1. Alkalinity as $CaCO_3$	mg/liter	p. 370	p. 143	p. 6,8
2. BOD_5	mg/liter	p. 489		
3. Chemical oxygen demand	mg/liter	p. 495	p. 219	p. 17
4. Total solids	mg/liter	p. 535		p. 280
5. Total dissolved solids	mg/liter			p. 275
6. Total suspended solids	mg/liter	p. 537		p. 278
7. Total volatile solids	mg/liter	p. 536		p. 282
8. Ammonia (as N)	mg/liter			p. 134,141
9. Kjeldahl (as N)	mg/liter	p. 469		p. 149,157
10. Nitrate (as N)	mg/liter	p. 458	p. 124	p. 170
		p. 461		p. 175
11. Total phosphorus	mg/liter	p. 526	p. 42	p. 235
		p. 532		p. 246,25
12. Acidity as $CaCO_3$	mg/liter		p. 148	
13. Total organic carbon	mg/liter	p. 257	p. 702	p. 221
14. Hardness (as CaCO3)	mg/liter	p. 179	p. 170	p. 76,78
15. Nitrite (as N)				p. 185
				p. 195
Analytical Methods for Trace Metals				
16. Aluminum	mg/liter	p. 210		p. 98
17. Antimony	mg/liter			
18. Arsenic	mg/liter	p. 65,62		p. 13
19. Barium	mg/liter	p. 210		
20. Beryllium	mg/liter	p. 67		
		p. 210		
21. Boron	mg/liter	p. 69		
22. Cadmium	mg/liter	p. 210	p. 692	p. 101
		p. 422		
23. Calcium	mg/liter	p. 84	p. 692	p. 102
24. Chromium (+6)	mg/liter	p. 429		p. 94

[1]*Standard Methods for the Examination of Water and Wastewater*, 13th Edition, 1971.

[2]*1972 Annual Book for ASTM Standards*; Water, Atmosphere, Part 23; American Society for Testing and Metals.

[3]*Methods for Chemical Analysis of Water and Wastes*, Environmental Protection Agency, 1971.

Parameters	Units	Approved Test Procedures: Standard Methods	ASTM	EPA Methods
25. Chromium (total)	mg/liter	p. 210 p. 426	p. 692 p. 403	p. 104
26. Cobalt	mg/liter		p. 692	
27. Copper	mg/liter	p. 210 p. 430	p. 692 p. 410	p. 106
28. Iron	mg/liter	p. 210 p. 433	p. 692 p. 152	p. 108
29. Lead	mg/liter	p. 210 p. 436	p. 692	p. 110
30. Magnesium	mg/liter	p. 210 p. 416	p. 692	p. 112
31. Manganese	mg/liter	p. 210	p. 692	p. 114
32. Mercury	mg/liter			
33. Molybdenum	mg/liter			
34. Nickel	mg/liter	p. 443	p. 692	
35. Potassium	mg/liter	p. 283 p. 285	p. 326	p. 115
36. Selenium	mg/liter			
37. Silver	mg/liter	p. 210		
38. Sodium	mg/liter	p. 317	p. 326	p. 118
39. Thallium	mg/liter			
40. Tin				
41. Titanium	mg/liter			
42. Vanadium	mg/liter	p. 157		
43. Zinc	mg/liter	p. 210 p. 444	p. 692	p. 120
Analytical Methods for Nutrients, Anions, and Organics				
44. Organic nitrogen	mg/liter	p. 468		p. 149
45. Ortho-phosphate (as P)	mg/liter	p. 532	p. 42	p. 235, 246, 259
46. Sulfate (as SO_4)	mg/liter	p. 331 p. 334	p. 51 p. 52	p. 286 p. 288
47. Sulfide (as S)	mg/liter	p. 551		p. 294
48. Sulfite (as SO_3)	mg/liter	p. 337	p. 261	
49. Bromide	mg/liter		p. 216	
50. Chloride	mg/liter	p. 96	p. 23	p. 29
51. Cyanide	mg/liter	p. 397	p. 556	p. 41
52. Fluoride	mg/liter	p. 171 p. 174	p. 191	p. 64
53. Chlorine	mg/liter	p. 382	p. 223	

Parameters	Units	Approved Test Procedures: Standard Methods	ASTM	EPA Methods
54. Oil and Grease	mg/liter	p. 254		
55. Phenols	mg/liter	p. 502	p. 445	p. 232
56. Surfactants	mg/liter	p. 339	p. 619	p. 131
57. Algicides*	mg/liter			
58. Benzidine	mg/liter	J. Assoc. Official Analy. Chem. 54:1383-1387, 1971		
59. Chlorinated organic comp.*	mg/liter			
60. Pesticides*	mg/liter			
Analytical Methods for Physical and Biological Properties				
61. Color platinum-cobalt units or dominant wavelength, hue, luminance, purity		p. 160 p. 392		p. 38
62. Specific conductance	mho/cm.	p. 323	p. 163	p. 284
63. Turbidity	jackson unit	p. 350	p. 467	p. 308
64. Fecal streptococci	number/ 100 ml.	p. 689 p. 690 p. 691		
65. Coliform (fecal)	number/ 100 ml.	p. 669 p. 684		
Radiological Parameters				
67. Alpha (total)	pCi/liter	p. 598	p. 509	
68. Alpha-counting error	pCi/liter	p. 598	p. 512	
69. Beta (total)	pCi/liter	p. 598	p. 478	
70. Beta-counting error	pCi/liter	p. 598	p. 478	
71. Radium (total)	pCi/liter	p. 611 p. 617	p. 674	

VI. Application for alternate test procedures:

A. Send application for approval of an alternative test procedure to the Regional Administrator.

1. Name and address of the responsible person or firm making the discharge (if not the applicant).

*Interim procedures prepared by the Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, Cincinnati, Ohio.

2. The applicable ID number of the existing or pending permit, issuing agency, and type of permit for which the alternative test procedure is requested.

3. The pollutant or parameter for which approval of an alternate testing procedure is being requested.

4. Justification for using the alternative testing procedures.

5. The detailed description of the proposed alternate test procedure with supporting data.

B. Within 90 days of receipt by the Regional Administrator of an application for an alternate test procedure, the Regional Administrator shall notify the applicant and the appropriate State agency of approval or rejection, or shall specify the additional information which is required to determine whether to approve the proposed test procedure.

SAMPLE PRESERVATION

I. Purposes

A. To inhibit bacterial growth: nutrients such as carbon sources, nitrogenous compounds and phosphorus compounds.

B. To prevent precipitation and adsorption to container: metals.

C. To prevent salt formation: acids and alkaline.

II. Preservatives use

A. Bacterial growth: $HgCl_2$, acid, refrigeration or freezing.

B. Precipitation: acid

C. Salt formation: acid for organic base, and alkali for cyanides and organic acid.

federal register

TUESDAY, OCTOBER 16, 1973
WASHINGTON, D.C.

Volume 38 ■ Number 199

PART II

ENVIRONMENTAL PROTECTION AGENCY

■

WATER PROGRAMS

Guidelines Establishing Test Procedures for Analysis of Pollutants

Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER D—WATER PROGRAMS

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

Notice was pubished in the FEDERAL REGISTER issue of June 29, 1973 (38 FR 17318) at 40 CFR 130, that the Environmental Protection Agency (EPA) was giving consideration to the testing procedures required pursuant to section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 (86 Stat. 816, et seq., Pub. L. 92–500 (1972)) hereinafter referred to as the Act. These considerations were given in the form of proposed guidelines establishing test procedures.

Section 304(g) of the Act requires that the Administrator shall promulgate guidelines establishing test procedures for the analysis of pollutants that shall include factors which must be provided in: 1, any certification pursuant to section 401 of the Act, or 2, any permit application pursuant to section 402 of the Act. Such test procedures are to be used by permit applicants to demonstrate that effluent discharges meet applicable pollutant discharge limitations, and by the States and other enforcement activities in routine or random monitoring of effluents to verify effectiveness of pollution control measures.

These guidelines require that discharge measurements, including but not limited to the pollutants and parameters listed in Table I, be performed by the test procedures indicated; or under certain circumstances by other test procedures for analysis that may be more advantageous to use, when such other test procedures have the approval of the Regional Administrator of the Region where such discharge will occur, and when the Director of an approved State National Pollutant Discharge Elimination System (NPDES) Program (hereinafter referred to as the Director) for the State in which such discharge will occur has no objection to such approval.

The list of test procedures in Table I is published herein as final rulemaking and represents major departures from the list of proposed test procedures which was published in 38 FR 17318, dated June 29, 1973. These revisions were made after carefully considering all written comments which were received pertaining to the proposed test procedures. All written comments are on file and available for public review with the Quality Assurance Division, Office of Research and Development, EPA, Washington, D.C.

The principal revisions to the proposed test procedures are as follows:

1. Where several reliable test procedures for analysis are available from the given references for a given pollutant or parameter, each such test procedure has been approved for use for making the measurements required by sections 401 and 402 and related sections of the Act. Approved test procedures have been selected to assure an acceptable level of intercomparability of pollutants discharge data. For several pollutants and parameters it has still been necessary to approve only a single test procedure to assure this level of acceptability. This is a major departure from the proposed test procedures which would have required the use of a single reference method for each pollutant or parameter.

2. Under certain circumstances a test procedure not shown on the approved list may be considered by an applicant to be more advantageous to use. Under guidelines in §§ 136.4 and 136.5 it may be approved by the Regional Administrator of the Region where the discharge will occur, providing the Director has no objections. Inasmuch as there is no longer a single approved reference method against which a comparison can be made, the procedures for establishing such comparisons that were required by the proposed test procedures in § 130.4(b) have been deleted from this final guideline for test procedures for the analysis of pollutants.

3. A mechanism is also provided to assure national uniformity of such approvals of alternate test procedures for the analysis of pollutants. This is achieved through a centralized, internal review within the EPA of all applications for the use of alternate testing procedures. These will be reviewed and approved or disapproved on the basis of submitted information and other available information and laboratory tests which may be required by the Regional Administrator.

As deemed necessary, the Administrator will expand or revise these guidelines to provide the most responsive and appropriate list of test procedures to meet the requirements of sections 304(g), 401 and 402 of the Act, as amended.

These final guidelines establishing test procedures for the analysis of pollutants supersede the interim list of test procedures published in the FEDERAL REGISTER on April 19, 1973 (38 FR 9740) at 40 CFR Part 126 and subsequent procedures published on July 24, 1973 (38 FR 19894) at 40 CFR Part 124. Those regulations established interim test procedures for the submittal of applications under section 402 of the Act. Because of the importance of these guidelines for test procedures for the analysis of pollutants to the National Pollution Discharge Elimination System (NPDES), the Administrator finds good cause to declare that these guidelines shall be effective October 16, 1973.

JOHN QUARLES,
Acting Administrator.

OCTOBER 3, 1973.

PART 136—TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

Sec.

AUTHORITY: Sec. 304(g) of Federal Water Pollution Control Act Amendments of 1972 86 Stat. 816, et seq., Pub. L. 92–500).

§ 136.1 Applicability.

The procedures prescribed herein shall, except as noted in § 136.5, be used to perform the measurements indicated whenever the waste constituent specified is required to be measured for:

(a) An application submitted to the Administrator, or to a State having an approved NPDES program, for a permit under section 402 of the Federal Water Pollution Control Act as amended (FWPCA), and,

(b) Reports required to be submitted by dischargers under the NPDES established by Parts 124 and 125 of this chapter, and,

(c) Certifications issued by States pursuant to section 401 of the FWPCA, as amended.

§ 136.2 Definitions.

As used in this part, the term:

(a) "Act" means the Federal Water Pollution Control Act, as amended, 33 U.S.C. 1314, et seq.

(b) "Administrator" means the Administrator of the U.S. Environmental Protection Agency.

(c) "Regional Administrator" means one of the EPA Regional Administrators.

(d) "Director" means the Director of the State Agency authorized to carry out an approved National Pollutant Discharge Elimination System Program under section 402 of the Act.

(e) "National Pollutant Discharge Elimination System (NPDES)" means the national system for the issuance of permits under section 402 of the Act and includes any State or interstate program which has been approved by the Administrator, in whole or in part, pursuant to section 402 of the Act.

(f) "Standard Methods" means *Standard Methods for the Examination of Water and Waste Water,* 13th Edition, 1971. This publication is available from the American Public Health Association, 1015 18th St. NW., Washington, D.C. 20036.

(g) "ASTM" means *Annual Book of Standards, Part 23, Water, Atmospheric Analysis, 1972.* This publication is available from the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pennsylvania 19103.

(h) "EPA Methods" means *Methods for Chemical Analysis of Water and Wastes,* 1971, Environmental Protection Agency, Analytical Quality Control Laboratory, Cincinnati, Ohio. This publication is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 (Stock Number 5501–0067).

§ 136.3 Identification of test procedures.

Every parameter or pollutant for which an effluent limitation is now specified pursuant to sections 401 and 402 of the Act is named together with test descriptions and references in Table I The discharge parameter values for which reports are required must be de-

termined by one of the standard analytical methods cited and described in Table I, or under certain circumstances by other methods that may be more advantageous to use when such other methods have been previously approved by the Regional Administrator of the Region in which the discharge will occur, and providing that the Director of the State in which such discharge will occur does not object to the use of such alternate test procedures.

Under certain circumstances the Regional Administrator or the Director in the Region or State where the discharge will occur may determine for a particular discharge that additional parameters or pollutants must be reported. Under such circumstances, additional test procedures for analysis of pollutants may be specified by the Regional Administrator or Director upon the recommendation of the Director of the Methods Development and Quality Assurance Research Laboratory.

TABLE I—LIST OF APPROVED TEST PROCEDURES

Parameter and units	Method	References		
		Standard methods	ASTM	EPA methods
General analytical methods:				
1. Alkalinity as $CaCO_3$ mg $CaCO_3$/liter.	Titration: electrometric, manual or automated method—methyl orange.	p. 370	p. 143	p. 6. p. 8.
2. B.O.D. five day mg/liter.	Modified winkler or probe method	p. 489		
3. Chemical oxygen demand (C.O.D.) mg/liter.	Dichromate reflux	p. 495	p. 219	p. 17.
4. Total solids mg/liter	Gravimetric 103–105° C	p. 535		p. 280.
5. Total dissolved (filterable) solids mg/liter.	Glass fiber filtration 180° C			p. 275.
6. Total suspended (nonfilterable) solids mg/liter.	Glass fiber filtration 103–105° C	p. 537		p. 278.
7. Total volatile solids mg/liter.	Gravimetric 550° C	p. 536		p. 282.
8. Ammonia (as N) mg/liter.	Distillation—nesslerization or titration automated phenolate.			p. 134. p. 141.
9. Kjeldahl nitrogen (as N) mg/liter.	Digestion + distillation—nesslerization or titration utomated digestion phenolate.	p. 469		p. 149. p. 157.
10. Nitrate (as N) mg/liter.	Cadmium reduction; brucine sulfate, automated cadmium or hydrazine reduction.	p. 158 p. 161	p. 124	p. 170. p. 175. p. 185.
11. Total phosphorus (as P) mg/liter.	Persulfate digestion and single reagent (ascorbic acid), or manual digestion, and automated single reagent or stannous chloride.	p. 526 p. 532	p. 42	p. 235. p. 246. p. 259.
12. Acidity mg $CaCO_3$/liter	Electrometric end point or phenolphthalein end point.		p. 148	
13. Total organic carbon (TOC) mg/liter.	Combustion—infrared method [1]	p. 257	p. 702	p. 221.
14. Hardness—total mg $CaCO_3$/liter.	EDTA titration; automated colorimetric atomic absorption.	p. 179	p. 170	p. 76. p. 78.
15. Nitrite (as N) mg/liter.	Manual or automated colorimetric diazotization.			p. 185. p. 195.
Analytical methods for trace metals:				
16. Aluminum—total [2] mg/liter.	Atomic absorption	p. 210		p. 98.
17. Antimony—total [2] mg/liter.	Atomic absorption [4]			
18. Arsenic—total mg/liter.	Digestion plus silver diethyldithiocarbamate; atomic absorption.[3]	p. 65 p. 62		p. 13.
19. Barium—total [2] mg/liter.	Atomic absorption [4]	p. 210		
20. Beryllium—total [2] mg/liter.	Aluminon; atomic absorption	p. 67 p. 210		
21. Boron—total mg/liter.	Curcumin	p. 69		
22. Cadmium—total [2] mg/liter.	Atomic absorption; colorimetric	p. 210 p. 422	p. 692	p. 101.
23. Calcium—total [2] mg/liter	EDTA titration; atomic absorption	p. 84	p. 692	p. 102.
24. Chromium VI mg/liter.	Extraction and atomic absorption; colorimetric.	p. 429		p. 34.
25. Chromium—total [2] mg/liter.	Atomic absorption; colorimetric	p. 210 p. 426	p. 692 p. 103	p. 104.
26. Cobalt—total [2] mg/liter.	Atomic absorption [4]		p. 392	
27. Copper—total [2] mg/liter.	Atomic absorption; colorimetric	p. 210 p. 430	p. 692 p. 410	p. 106.
28. Iron—total [2] mg/liter	do	p. 210 p. 433	p. 692 p. 152	p. 108.
29. Lead—total [2] mg/liter	do	p. 210 p. 436	p. 692	p. 110.
30. Magnesium—total [2] mg/liter.	Atomic absorption; Gravimetric	p. 210 p. 416 p. 201	p. 692	p. 112.
31. Manganese—total [2] mg/liter.	Atomic absorption	p. 210	p. 692	p. 114.
32. Mercury—total mg/liter.	Flameless atomic absorption [5]			
33. Molybdenum—total [2] mg/liter.	Atomic absorption [4]			
34. Nickel—total [2] mg/liter.	Atomic absorption; colorimetric [4]	p. 443	p. 692	
35. Potassium—total [2] mg/liter.	Atomic absorption; colorimetric; flame photometric.	p. 283 p. 285	p. 326	p. 115.
36. Selenium—total mg/liter.	Atomic absorption [3]			
37. Silver—total [2]	Atomic absorption [4]	p. 210		
38. Sodium—total [2] mg/liter.	Flame photometric; atomic absorption	p. 317	p. 326	p. 118.
39. Thallium—total [2] mg/liter.	Atomic absorption [4]			
40. Tin—total [2] mg/liter	do			
41. Titanium—total mg/liter.	do			
42. Vanadium—total [2] mg/liter.	Atomic Absorption; [4] Colorimetric	p. 157		
43. Zinc—total [2] mg/liter	Atomic Absorption; Colorimetric	p. 210 p. 444	p. 692	p. 120.
Analytical methods for nutrients, anions, and organics:				
44. Organic nitrogen (as N) mg/liter.	Kjeldahl nitrogen minus ammonia nitrogen.	p. 468		p. 140.
45. Ortho-phosphate (as P) mg/liter.	Direct single reagent; automated single reagent or stannous chloride.	p. 532	p. 42	p. 235. p. 246. p. 259.
46. Sulfate (as SO_4) mg/liter.	Gravimetric; turbidimetric; automated colorimetric—barium chloranilate.	p. 331 p. 334	p. 51 p. 52	p. 286. p. 288.
47. Sulfide (as S) mg/liter.	Titrimetric—iodine	p. 551		p. 294.
48. Sulfite (as SO_3) mg/liter.	Titrimetric; iodide-iodate	p. 337	p. 261	
49. Bromide mg/liter	do		p. 216	
50. Chloride mg/liter	Silver nitrate; mercuric nitrate; automated colorimetric-ferricyanide.	p. 96 p. 97	p. 23 p. 21	p. 29. p. 31.
51. Cyanide—total mg/liter.	Distillation—silver nitrate titration or pyridine pyrazolone colorimetric.	p. 397	p. 556	p. 41.
52. Fluoride mg/liter	Distillation—SPADNS	p. 171 p. 174	p. 191	p. 64.
53. Chlorine—total residual mg/liter.	Colorimetric; amperometric titration	p. 382	p. 223	
54. Oil and grease mg/liter.	Liquid-Liquid extraction with trichlorotrifluoroethane.	p. 264		
55. Phenols mg/liter	Colorimetric, [4] AAP	p. 502	p. 445	p. 232.
56. Surfactants mg/liter	Methylene blue colorimetric	p. 339	p. 619	p. 131.
57. Algicides mg/liter	Gas chromatography [6]			
58. Benzidine mg/liter	Diazotization—colorimetric [7]			
59. Chlorinated organic compounds (except pesticides) mg/liter.	Gas chromatography [8]			
60. Pesticides mg/liter	Gas chromatography [8]			
Analytical methods for physical and biological parameters:				
61. Color platinum-cobalt units or dominant wave-length, hue, luminance, purity.	Colorimetric; spectrophotometric	p. 160 p. 392		p. 38.
62. Specific conductance mho/cm at 25° C.	Wheatstone bridge	p. 323	p. 168	p. 284.
63. Turbidity jackson units.	Turbidimeter	p. 350	p. 167	p. 308.

See Note at end of Table I

Parameter and units	Method	References		
		Standard methods	ASTM	EPA methods
64. Fecal streptococci bacteria number/100 ml.	MPN; membrane filter; plate count	p. 689 p. 690 p. 691		
65. Coliform bacteria (fecal) number/100 ml.	MPN: Membrane filter	p. 660 p. 684		
66. Coliform bacteria (total) number/100 ml.	do	p. 664 p. 679		
Radiological parameters:				
67. Alpha—total pCi/liter.	Proportional counter; scintillation counter	p. 598	p. 509	
68. Alpha—counting error pCi/liter.	do	p. 598	p. 512	
69. Beta—total pCi/liter	Proportional counter†	p. 598	p. 478	
70. Beta—counting error pCi/liter.	do	p. 598	p. 478	
71. Radium—total pCi/liter.	Proportional counter; scintillation counter	p. 611 p. 617	p. 674	

[1] A number of such systems manufactured by various companies are considered to be comparable in their performance. In addition, another technique, based on Combustion-Methane Detection, is also acceptable.

[2] For the determination of total metals the sample is not filtered before processing. Choose a volume of sample appropriate for the expected level of metals. If much suspended material is present, as little as 50–100 ml of well-mixed sample will most probably be sufficient. (The sample volume required may also vary proportionally with the number of metals to be determined.)

Transfer a representative aliquot of the well-mixed sample to a Griffin beaker and add 3 ml of concentrated distilled HNO_3. Place the beaker on a hotplate and evaporate to dryness making certain that the sample does not boil. Cool the beaker and add another 3 ml portion of distilled concentrated HNO_3. Cover the beaker with a watch glass and return to the hotplate. Increase the temperature of the hotplate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary until the digestion is complete, generally indicated by a light colored residue. Add (1:1 with distilled water) distilled concentrated HCl in an amount sufficient to dissolve the residue upon warming. Wash down the beaker walls and the watch glass with distilled water and filter the sample to remove silicates and other insoluble material that could clog the atomizer. Adjust the volume to some predetermined value based on the expected metal concentrations. The sample is now ready for analysis. Concentrations so determined shall be reported as "total".

[3] See D. C. Manning, "Technical Notes", Atomic Absorption Newsletter, Vol. 10, No. 6 p. 123, 1971. Available from Perkin-Elmer Corporation, Main Avenue, Norwalk, Connecticut 06852.

[4] Atomic absorption method available from Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, USEPA, Cincinnati, Ohio 45268.

[5] For updated method, see: Journal of the American Water Works Association 64, No. 1, pp. 20–25 (Jan. 1972) or ASTM Method D 3223–73, American Society for Testing and Materials Headquarters, 1916 Race St., Philadelphia, Pa. 19103.

[6] Interim procedures for algicides, chlorinated organic compounds, and pesticides can be obtained from the Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, USEPA, Cincinnati, Ohio 45268.

[7] Benzidine may be estimated by the method of M.A. El-Dib, "Colorimetric Determination of Aniline Derivatives in Natural Waters", El-Dib, M.A., Journal of the Association of Official Analytical Chemists, Vol. 54, No. 6, Nov., 1971, pp. 1383–1387.

†As a prescreening measurement.

§ 136.4 Application for alternate test procedures.

(a) Any person may apply to the Regional Administrator in the Region where the discharge occurs for approval of an alternative test procedure.

(b) When the discharge for which an alternative test procedure is proposed occurs within a State having a permit program approved pursuant to section 402 of the Act, the applicant shall submit his application to the Regional Administrator through the Director of the State agency having responsibility for issuance of NPDES permits within such State.

(c) Unless and until printed application forms are made available, an application for an alternate test procedure may be made by letter in triplicate. Any application for an alternate test procedure under this subchapter shall:

(1) Provide the name and address of the responsible person or firm making the discharge (if not the applicant) and the applicable ID number of the existing or pending permit, issuing agency, and type of permit for which the alternate test procedure is requested, and the discharge serial number.

(2) Identify the pollutant or parameter for which approval of an alternate testing procedure is being requested.

(3) Provide justification for using testing procedures other than those specified in Table I.

(4) Provide a detailed description of the proposed alternate test procedure, together with references to published studies of the applicability of the alternate test procedure to the effluents in question.

§ 136.5 Approval of alternate test procedures.

(a) The Regional Administrator of the region in which the discharge will occur has final responsibility for approval of any alternate test procedure.

(b) Within thirty days of receipt of an application, the Director will forward such application, together with his recommendations, to the Regional Administrator. Where the Director recommends rejection of the application for scientific and technical reasons which he provides, the Regional Administrator shall deny the application, and shall forward a copy of the rejected application and his decision to the Director of the State Permit Program and to the Director of the Methods Development and Quality Assurance Research Laboratory.

(c) Before approving any application for an alternate test procedure, the Regional Administrator shall forward a copy of the application to the Director of the Methods Development and Quality Assurance Laboratory for review and recommendation.

(d) Within ninety days of receipt by the Regional Administrator of an application for an alternate test procedure, the Regional Administrator shall notify the applicant and the appropriate State agency of approval or rejection, or shall specify the additional information which is required to determine whether to approve the proposed test procedure. Prior to the expiration of such ninety day period, a recommendation providing the scientific and other technical basis for acceptance or rejection will be forwarded to the Regional Administrator by the Director of the Methods Development and Quality Assurance Research Laboratory. A copy of all approval and rejection notifications will be forwarded to the Director, Methods Development and Quality Assurance Research Laboratory, for the purposes of national coordination.

[FR Doc.73–21466 Filed 10–15–73;8:45 am]

1. METHOD FOR ORGANOCHLORINE PESTICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of various organochlorine pesticides, including some pesticidal degradation products and related compounds in industrial effluents. Such compounds are composed of carbon, hydrogen, and chlorine, but may also contain oxygen, sulfur, phosphorus, nitrogen or other halogens.

1.2 The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, pentachloronitrobenzene and trifluralin. Under favorable circumstances, Strobane, toxaphene, chlordane (tech.) and others may also be determined. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

1.3 When organochlorine pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

2. Summary

2.1 The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and/or the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography

and liquid-liquid partition, methods for elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography. Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4 and 1.5, (1).

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organochlorine pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organochlorine pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Polychlorinated Biphenyls (PCB's) - Special attention is called to industrial plasticizers and hydraulic fluids such as the PCB's which are a potential source of interference in pesticide analysis. The presence of PCB's is indicated by a large number of partially resolved or unresolved peaks which may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures (2,3).

3.4 Phthalate Esters - These compounds, widely used as plasticizers, respond to the electron capture detector and are a source of interference in the determination of organochlorine pesticides using this detector. Water leaches these materials from plastics, such as polyethylene bottles and tygon tubing. The presence of phthalate esters is implicated in samples that respond to electron capture but not to the microcoulometric or electrolytic conductivity halogen detectors or to the flame photometric detector.

3.5 Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, eg, parathion, also respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. Such compounds can be identified by their response to the flame photometric detector (4).

4. Apparatus and Materials

4.1 Gas Chromatograph Equipped with glass lined injection port.

4.2 Detector Options:

4.2.1 Electron Capture Radioactive (tritium or nickel 63)

4.2.2 Microcoulometric Titration

4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing - Pyrex (180 cm long x 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom Q (100-120 mesh)

4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.

4.4.4.1 OV-1, 3%

4.4.4.2 OV-210, 5%

4.4.4.3 OV-17, 1.5% plus QF-1, 1.95%

4.4.4.4 QF-1, 6% plus SE-30, 4%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (macro) and two ball (micro)

4.5.2 Evaporative Flasks - 500 ml

4.5.3 Receiver Ampuls - 10 ml, graduated

4.5.4 Ampul Stoppers

4.6 Chromatographic Column Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50 and 100 µl

4.9 Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Graduated cylinders - 100 and 250 ml

4.12 Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

5. Reagents, Solvents, and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).

5.4 Sodium Hydroxide (ACS) 10 N in distilled water.

5.5 Sodium Sulfate - (ACS) Granular, anhydrous.

5.6 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.7.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.7.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.7.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.8 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - nanograde, redistill in glass if necessary

5.9 Pesticide Standards - Reference grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when $\leq$ 0.06 ng is injected for electron capture detection and $\leq$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.

6.3 The elution order and retention ratios of various organochlorine pesticides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended (5).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For a sensitivity requirement of 1 µg/l, when using microcoulometric **or electrolytic** conductivity methods for detection take 100 ml of sample for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

8.3 Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Analyze by gas chromatography unless a need for cleanup is indicated. (See Section 10).

10. Clean-up and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides. Of the pesticides listed in Scope (1.2) only mirex is not efficiently recovered.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100 ml portions of

hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

10.2.4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition 10.4).

Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

10.3.5 Analyze by gas chromatography.

10.4 Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin	DDT	Pentachloro-nitrobenzene
BHC	Heptachlor	
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin
	Mirex	PCB's

15% Eluate	50% Eluate
Endosulfan I	Endosulfan II
Endrin	Captan
Dieldrin	
Dichloran	
Phthalate esters	

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

11. Calculation of Results

11.1 Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2. (1).

(1) $$\text{Micrograms/liter} = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

$A = \frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

V_s = Volume of water extracted (ml)

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

REFERENCES

1. "Method for Organic Pesticides in Water and Wastewater," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268, 1971.

2. Monsanto Methodology for Aroclors - Analysis of Environmental Materials for Biphenyls, Analytical Chemistry Method 71-35, Monsanto Company, St. Louis, Missouri 63166, 1970.

3. "Method for Polychlorinated Biphenyls in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268, 1973.

4. "Method for Organophosphorus Pesticides in Industrial Effluents," Environmental Protection Agency, National Environmental Research Center, Cincinnati Ohio 45268, 1973.

5. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

6. "Pesticide Analytical Manual," U.S. Dept. of Health, Education and Welfare, Food and Drug Administration, Washington, D.C.

7. "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Perrine Primate Research Laboratories, Perrine, Florida 33157, 1971.

8. Mills, P.A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).

9. Goerlitz, D.F. and Brown, E., "Methods for Analysis of Organic Substances in Water," Techniques of Water Resources Investigations of the United States Geological Survey, Book 5, Chapter A3, U.S. Department of the Interior, Geological Survey, Washington, D.C. 20402, 1972, pp. 24-40.

10. Steere, N.V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

Table 1

RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase[1]	1.5% OV-17 + 2.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200 C	180 C	180 C	200 C
Argon/Methane Carrier Flow	60 ml/min	70 ml/min	70 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Trifluralin	0.39	1.11	0.33	0.57
∝-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p'-DDT	3.16	2.70	2.69	2.39
p,p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

[1] All columns glass, 180 cm X 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

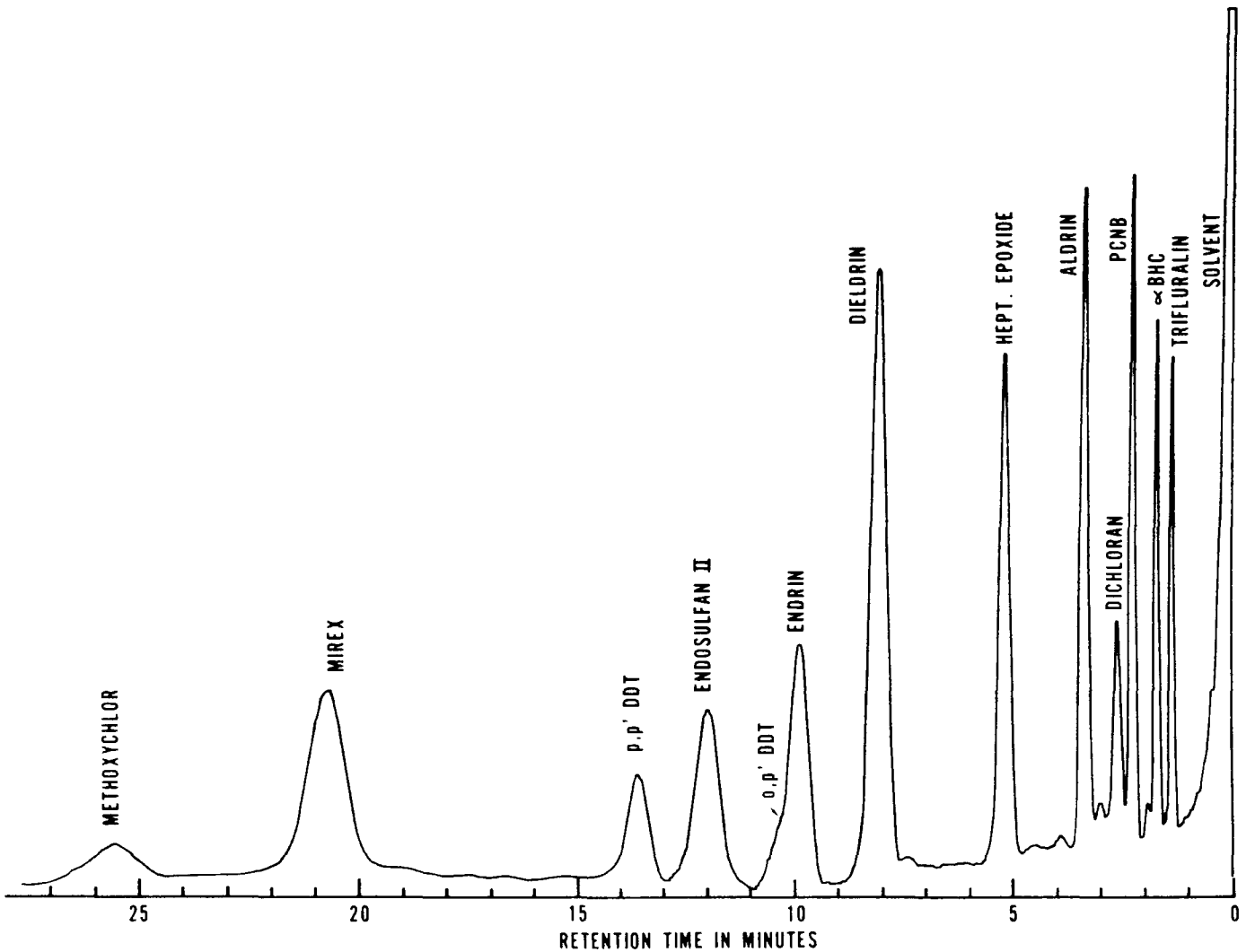


Figure 1. Column Packing: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

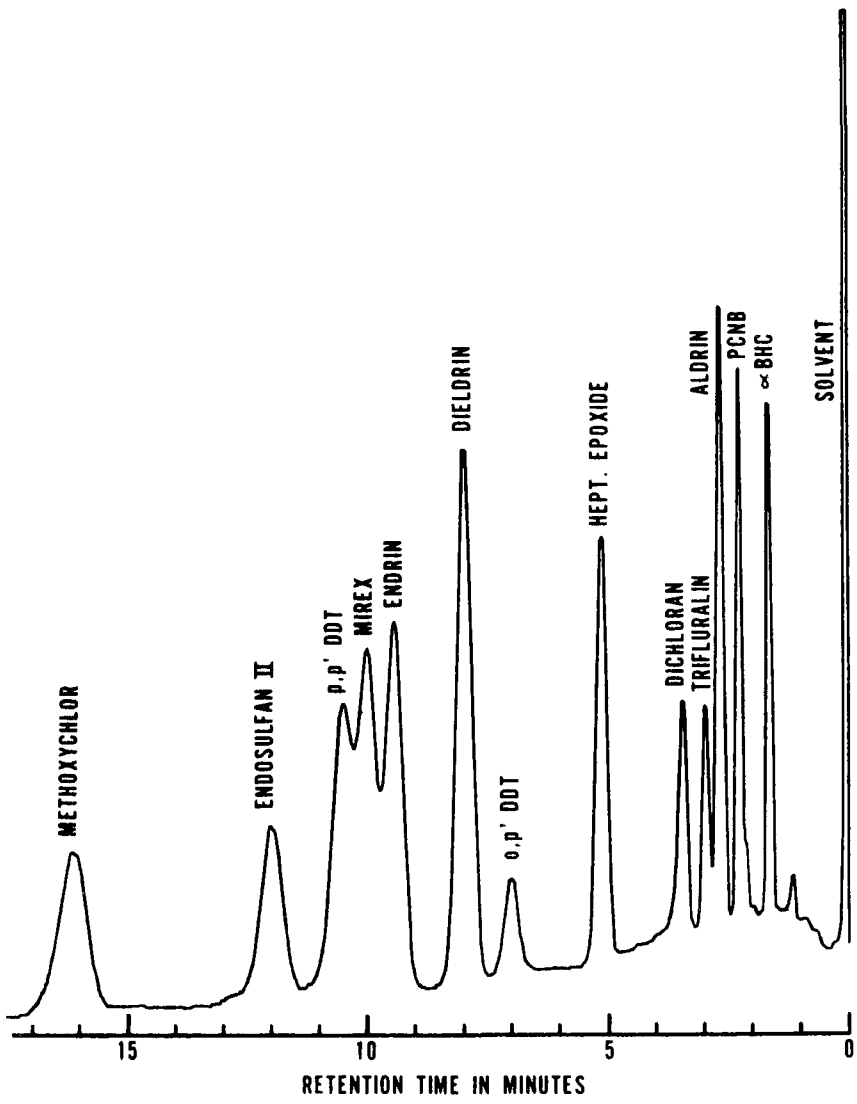


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

☆ U S GOVERNMENT PRINTING OFFICE: 1973— 759-555/1145

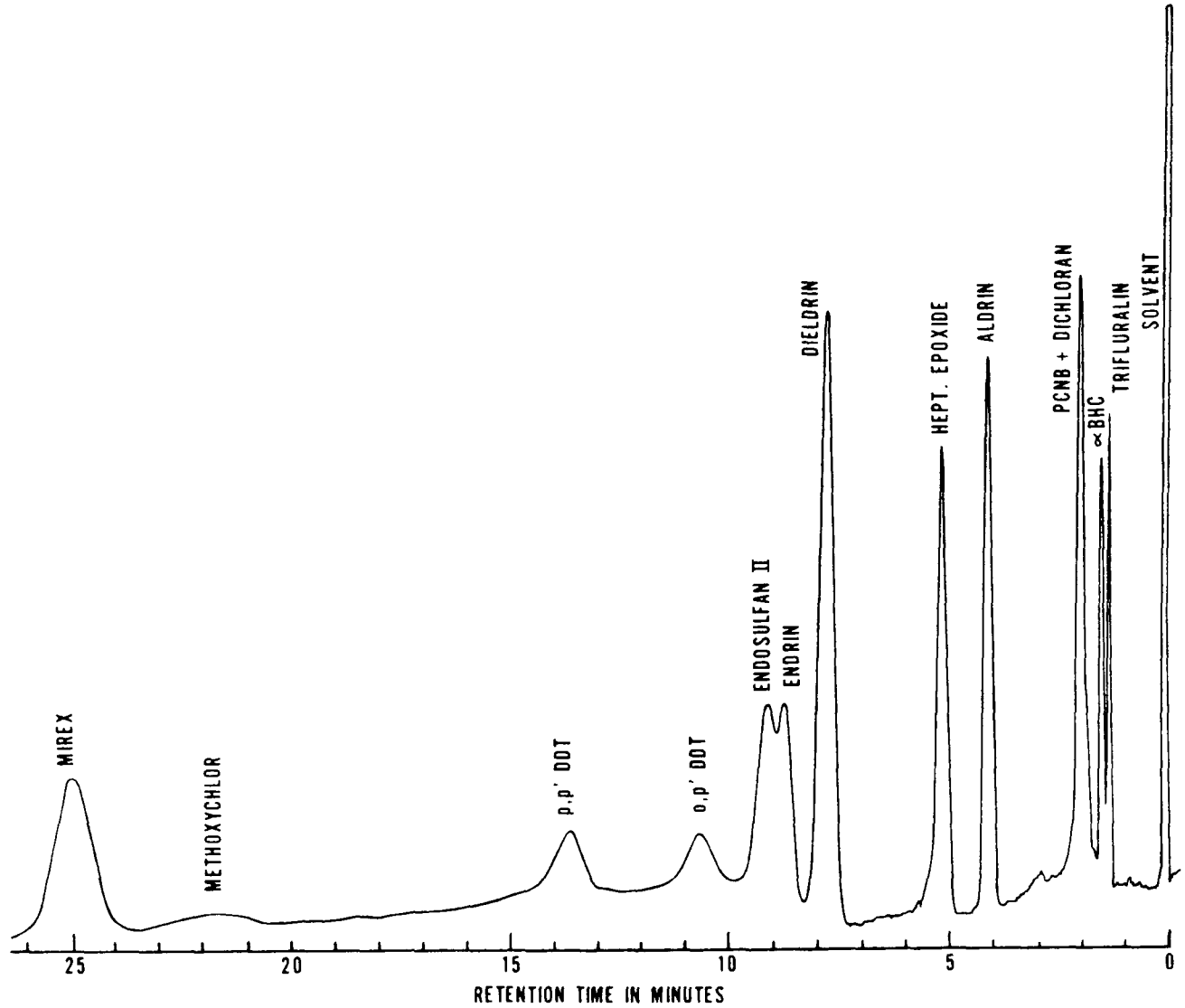


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Argon/Methane at 70 ml/min, Column Temperature: 180 C, Detector: Electron Capture.

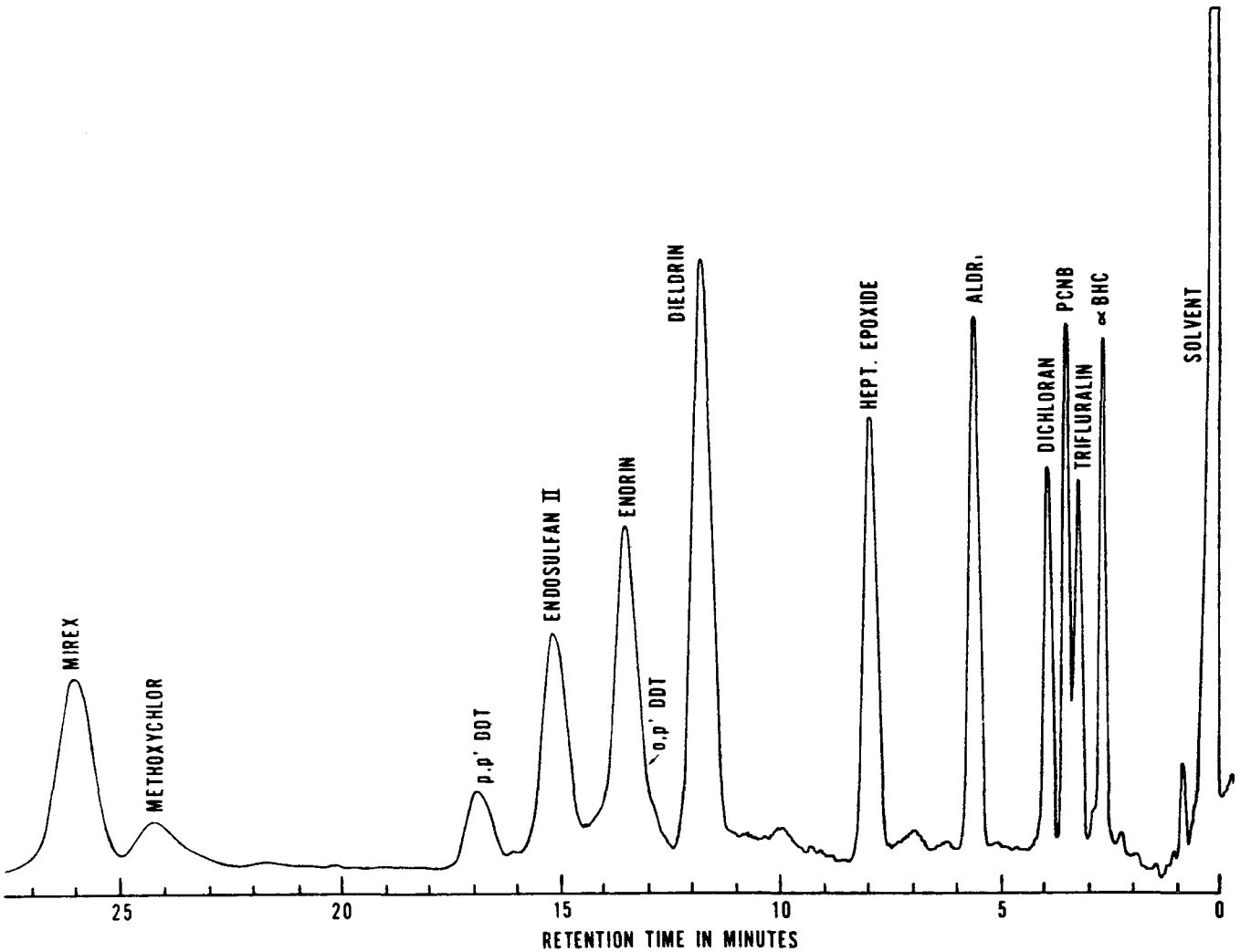


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Argon/Methane at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

2. METHOD FOR ORGANOPHOSPHORUS PESTICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of various organophosphorus pesticides and may be extended to pesticidal degradation products and related compounds. Such compounds are composed of carbon, hydrogen, and phosphorus, but may also contain sulfur, oxygen, halogen or nitrogen.

1.2 The following compounds may be determined individually by this method with a sensitivity of 1 µg/l: Disyston, Diazinon, malathion, Methyl Parathion, Parathion, demeton, and Guthion. Under favorable circumstances other organophosphorus pesticides may also be determined. However, the usefulness of the method for other specific compounds must be demonstrated by the analyst before applying it to sample analysis.

1.3 When organophosphorus pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

2. Summary

2.1 The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography

and liquid-liquid partition, methods for the elimination of non-pesticide interferences and the preseparation of pesticide mixtures. Identification is made by selective gas chromatographic separation and may be corroborated through the use of two or more unlike columns. Detection and measurement are best accomplished by flame photometric gas chromatography using a phosphorus specific filter. The electron capture detector, though non-specific, may also be used for those compounds to which it responds. Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part 1, Sections 1.4, 1.5 (1).

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organophosphorus pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organophosphorus pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures

for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Compounds such as organochlorine pesticides, polychlorinated biphenyls and phthalate esters interfere with the analysis of organophosphorus pesticides by electron capture gas chromatography. When encountered these interferences are overcome by the use of the phosphorus specific flame photometric detector. If such a detector is not available, these interferences may be removed from the sample by using the clean-up procedures described in the EPA Methods for those compounds (2) (3).

3.4 Elemental sulfur will interfere with the determination of organophosphorus pesticides by flame photometric and electron capture gas chromatography. The elimination of elemental sulfur as an interference is described in Section 10.5, Clean-up and Separation Procedures.

4. Apparatus and Materials

4.1 Gas Chromatograph Equipped with glass lined injection port.

4.2 Detector Options:

4.2.1 Flame Photometric - 526 mμ phosphorus filter.

4.2.2 Electron Capture - Radioactive (tritium or nickel-63)

4.3 Recorder Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing Pyrex (180 cm long x 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom Q (100-120 mesh)

4.4.4 Liquid Phases Expressed as weight percent coated on solid support.

4.4.4.1 OV-1, 3%

4.4.4.2 OV-210, 5%

4.4.4.3 OV-17, 1.5% plus QF-1, 1.95%

4.4.4.4 QF-1, 6% plus SE-30, 4%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (macro) and two ball (micro)

4.5.2 Evaporative Flasks - 500 ml

4.5.3 Receiver Ampuls - 10 ml, graduated

4.5.4 Ampul Stoppers

4.6 Chromatographic Column - Chromaflex (400 mm x 19 mm ID) with coarse fritted plate and Teflon stopcock on bottom; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column Pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50 and 100 µl

4.9 Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

4.10 Micro-pipets - disposable (140 mm long x 5 mm ID)

4.11 Blender - High speed, glass or stainless steel cup.

4.12 Graduated cylinders - 100 and 250 ml

4.13 Florisil PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

4.14 Alumina - Woelm, neutral; deactivate by pipeting 1 ml of distilled water into 125 ml ground glass-stoppered Erlenmeyer flask. Rotate flask to distribute water over surface of glass. Immediately add 19.0 g fresh alumina through small powder funnel. Shake flask containing mixture for two hours on a mechanical shaker (4).

5. Reagents, Solvents, and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.4 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.5 Sodium Sulfate - (ACS) Granular, anhydrous.

5.6 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.7.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.7.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.7.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.8 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - Nanograde, redistill in glass if necessary.

5.9 Pesticide Standards - Reference Grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when $\leq$ 1.5 ng is injected for flame photometric detection and $\leq$ 0.06 ng is injected for electron capture detection. For all quantitative measurements the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.

6.3 The elution order and retention ratios of various organophosphorus pesticides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended (5).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample, if suspended matter is present, and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For a sensitivity requirement of 1 µg/l, when using flame photometric or electron capture for detection, take 100 ml of sample for analysis.

8.3 Quantitatively transfer a 100 ml aliquot of sample into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent, add the solvent to the separatory funnel, and complete the extraction procedure a second time Perform a third extraction in the same manner.

9.3 Concentrate the extract in the K-D evaporator on a hot water bath.

9.4 Analyze by gas chromatography unless a need for cleanup is indicated. (See Section 10).

10. Clean-up and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer

through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

10.2.4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

10.2.5 Analyze by gas chromatography unless a need for further clean-up is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to four eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition, 10.4.) Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution

with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.3.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

10.3.5 Analyze by gas chromatography.

10.4 Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric-acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate	15% Eluate
Demeton	Diazinon
Disyston	Malathion (trace)
	Methyl Parathion
50% Eluate	**100% Eluate**
Malathion	Guthion (80%)
Guthion (20%)	

For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

10.5 Removal of Sulfur - If elemental sulfur interferes with the gas chromatographic analysis, it can be removed by the use of an alumina microcolumn.

10.5.1 Adjust the sample extract volume to 0.5 ml in a K-D apparatus, using a two-ball Snyder microcolumn.

10.5.2 Plug a disposable pipet with a small quantity of glass wool. Add enough alumina to produce a 3 cm column after settling. Top the alumina with a 0.5 cm layer of anhydrous sodium sulfate.

10.5.3 Quantitatively transfer the concentrated extract to the alumina microcolumn using a 100 µl syringe. Rinse the

ampul with 200 µl of hexane and add to the microcolumn.

10.5.4 Elute the microcolumn with 3 ml of hexane and discard the first eluate which contains the elemental sulfur.

10.5.5 Next elute the column with 5 ml of 10% hexane in methylene chloride. Collect the eluate in a 10 ml graduated ampul.

10.5.6 Analyze by gas chromatography.

NOTE: If the electron capture detector is to be used methylene chloride must be removed. To do this, attach the ampul to a K-D apparatus (500 ml flask and 3-ball Snyder column) and concentrate to about 0.5 ml. Adjust volume as required prior to analysis.

11. Calculation of Results

11.1 Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2.(1).

(1) $$\text{Micrograms/liter} = \frac{(A)\ (B)\ (V_t)}{(V_i)\ (V_s)}$$

$A = \frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

V_s = Volume of water extracted (ml)

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

TABLE 1

RETENTION TIMES OF SOME ORGANOPHOSPHOROUS PESTICIDES
RELATIVE TO PARATHION

Liquid Phase[1]	1.5% OV-17 + 1.95% QF-1	6% QF-1 + 4% SE-30	5% OV-210	7% OV-1
Column Temp.	215 C	215 C	200 C	200 C
Nitrogen Carrier Flow	70 ml/min	70 ml/min	60 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Naled	with solvent	0.11 0.15	with solvent	with solvent
DDVP	0.16	0.16	0.13	0.29
Phosdrin	0.26	0.24	0.23	0.36
Demeton[2]	0.46	0.26 0.43	0.20 0.38	0.74
Thimet	0.35	0.35	0.23	0.47
Diazinon	0.40	0.38	0.25	0.59
Disulfoton	0.46	0.45	0.31	0.62
Dimethoate	0.65	0.57	0.58	0.72
Ronnel	0.65	0.60	0.43	0.83
Merphos	0.69	0.67	0.34	1.23
Malathion	0.86	0.78	0.73	0.92
Methyl Parathion	0.82	0.80	0.81	0.79
Parathion	1.00	1.00	1.00	1.00
Phosphamidon[2]	0.98	1.06	1.30	0.87
DEF	1.25	1.12	0.78	1.78
Ethion	2.04	1.58	2.27	2.26
Trithion	2.21	1.66	1.18	2.57
EPN	4.23	3.32	3.37	3.84
Guthion	6.65	4.15	4.44	4.68
Parathion (min absolute)	4.5	6.6	5.7	3.1

[1]All columns glass, 180 xm x 4 mm ID, solid support Gas-Chrom Q, 100/120 mesh.

[2]Anomalous, multipeak response often encountered.

REFERENCES

(1) "Methods for Organic Pesticides in Water and Wastewater," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(2) "Method for Organochlorine Pesticides in Industrial Effluents," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(3) "Method for Polychlorinated Biphenyls (PCB's) in Industrial Effluents," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(4) Law, L. M. and Goerlitz, D. F., "Microcolumn Chromatographic Clean-up for the Analysis of Pesticides in Water," Journal of the Association of Official Analytical Chemists, 53, 1276 (1970).

(5) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(6) "Pesticide Analytical Manual," U.S. Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.

(7) "Analysis of Pesticide Residues in Human and Environmental Samples," U.S. Environmental Protection Agency, Perrine Primate Research Laboratories, Perrine, Florida 33157, 1971.

(8) Mills, P. A., "Variation of Florisil Activity: Simple Method for Measuring Adsorbent Capacity and Its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).

(9) Goerlitz, D. F. and Brown, E., "Method for Analysis of Organic Substances in Water'" Techniques of Water Resources Investigations of the United States Geological Survey, Book 5, Chapter A3, U.S. Department of the Interior, Geological Survey, Washington, D.C. 20402, 1972, pp. 24-40.

(10) Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

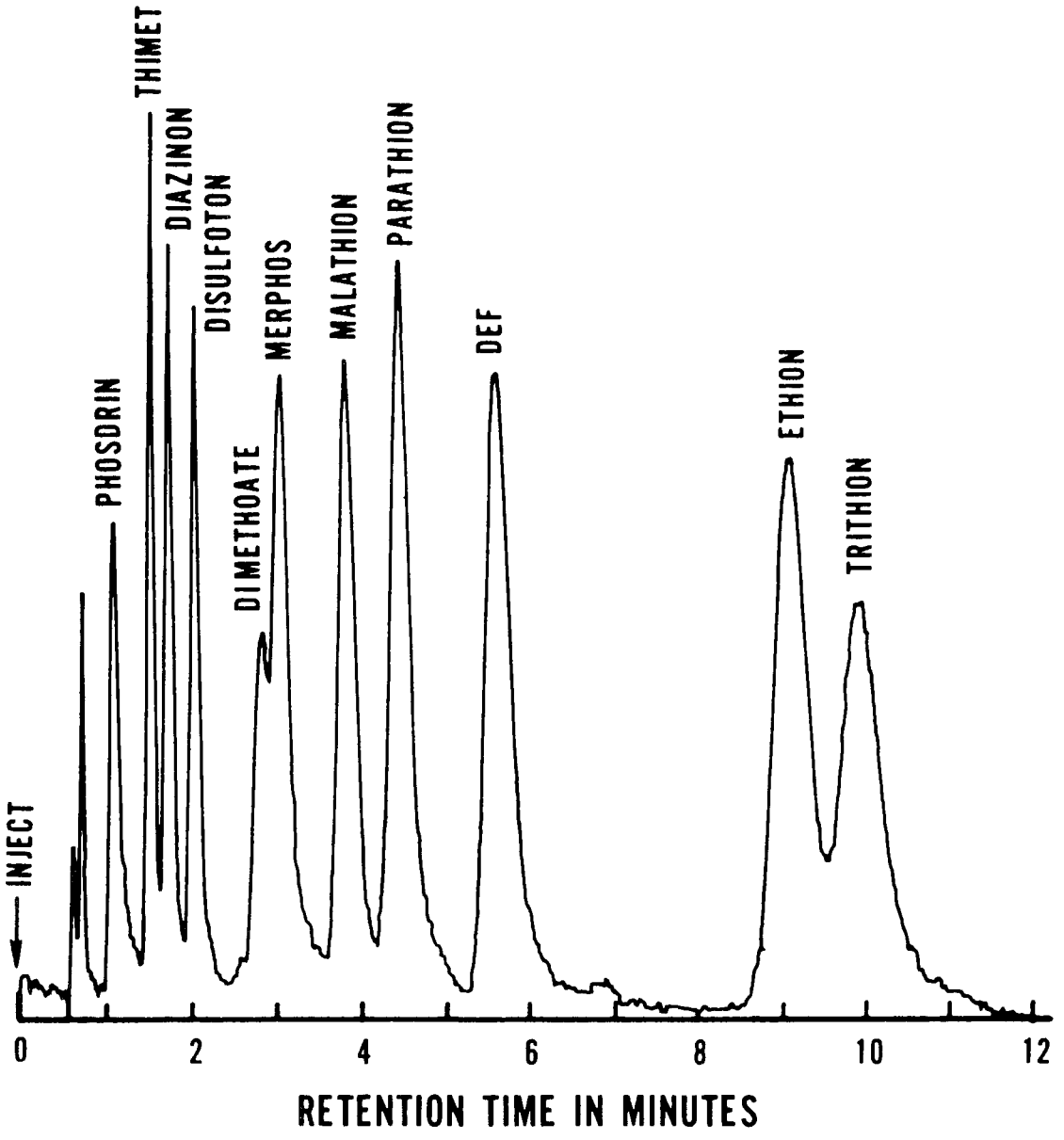


Figure 1. Column Packing: 1.5% OV-17 + 1.95 % QF-1, Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 215 C, Detector: Flame Photometric (Phosphorus).

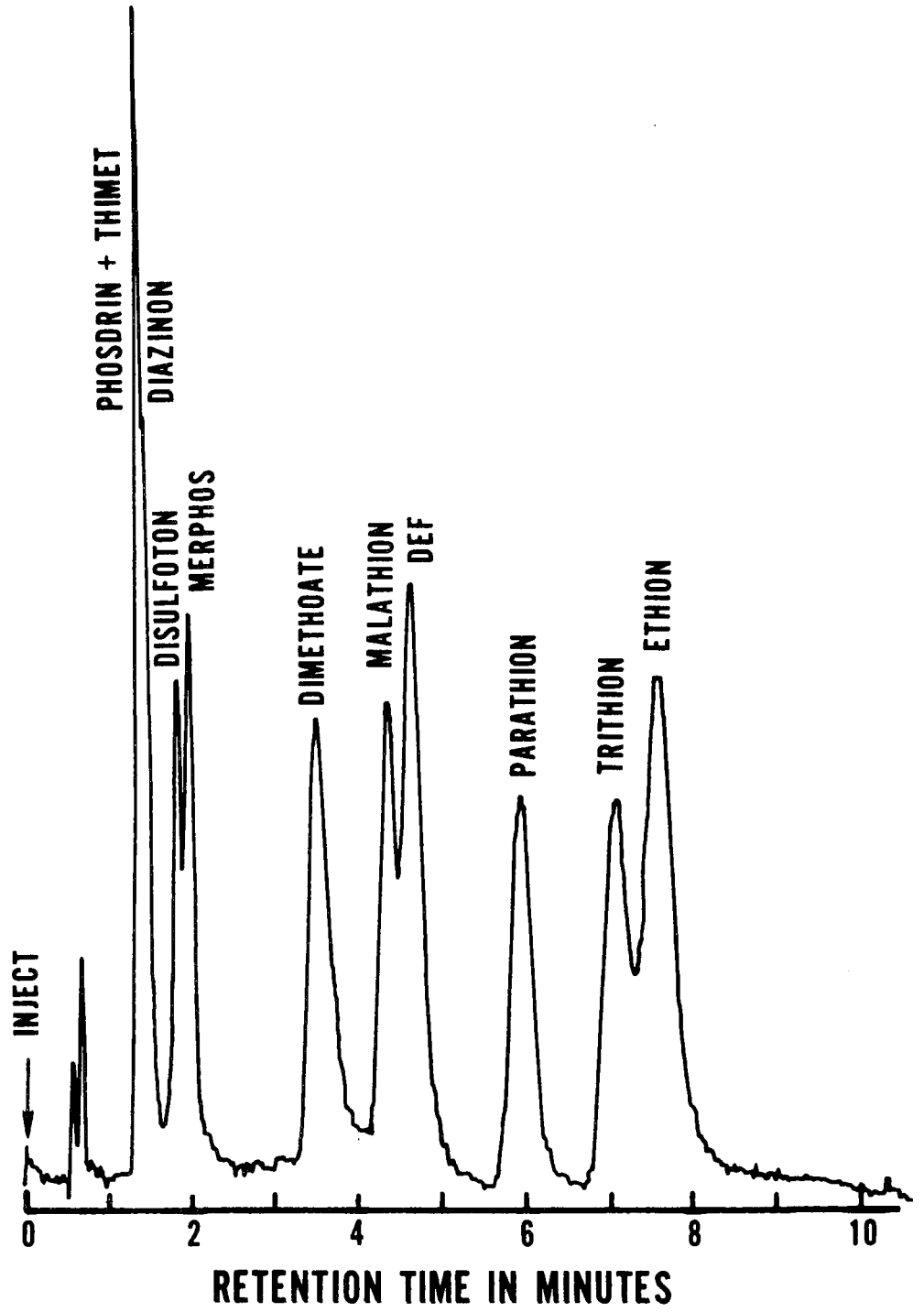


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

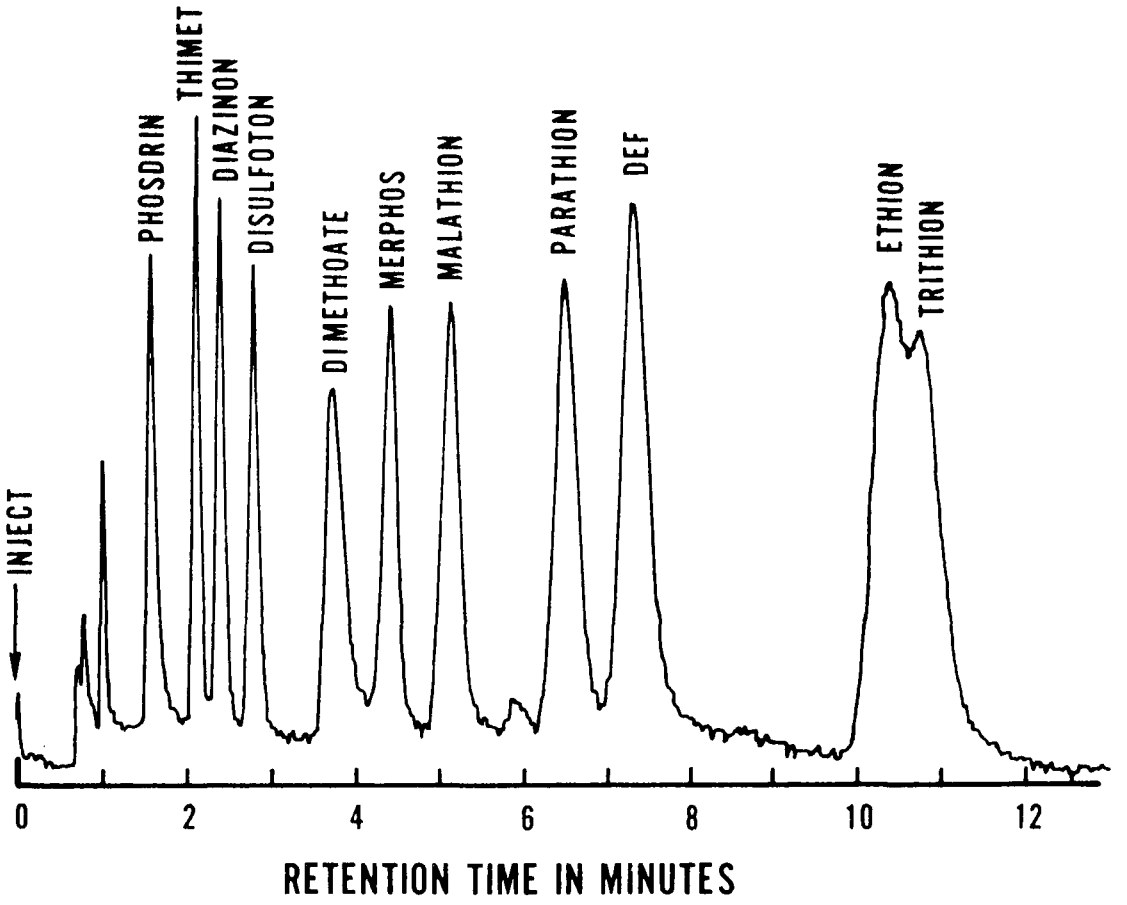


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 125 C, Detector: Flame Photometric (Phosphorus).

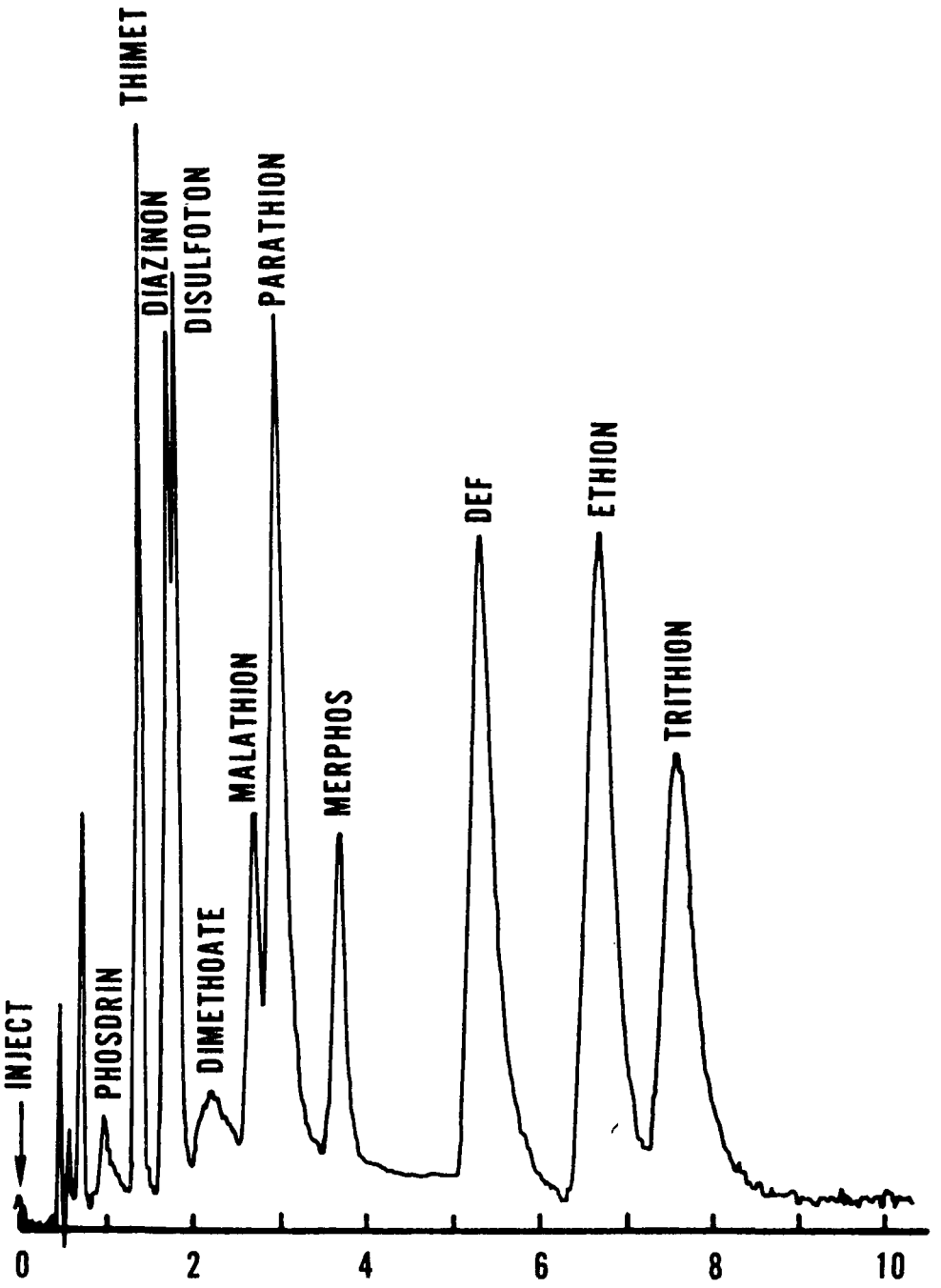


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1146

3. METHOD FOR POLYCHLORINATED BIPHENYLS [PCB'S] IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of certain polychlorinated biphenyl (PCB) mixtures including: Aroclors 1221, 1232, 1242, 1248, 1254, 1260 and 1016.

1.2 The method is an extension of the method for organochlorine pesticides in industrial effluents (1). It is designed so that determination of both the PCB's and the organochlorine pesticides may be made on the same sample.

1.3 The limit of detection is approximately 1 µg/l for each Aroclor mixture.

2. Summary

2.1 The PCB's and the organochlorine pesticides are co-extracted by liquid-liquid extraction and, insofar as possible, the two classes of compounds separated from one another prior to gas chromatographic determination. A combination of the standard Florisil column cleanup procedure and a silica gel microcolumn separation procedure (2)(3) are employed. Identification is made from gas chromatographic patterns obtained through the use of two or more unlike columns. Detection and measurement is accomplished using an electron capture, microcoulometric, or electrolytic conductivity detector. Techniques for confirming qualitative identification are suggested.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer

to (4), Part I, Sections 1.4 and 1.5.

3.2 The interferences in industrial effluents are high and varied and pose great difficulty in obtaining accurate and precise measurement of PCB's and organochlorine pesticides. Separation and cleanup procedures are generally required to eliminate these interferences; however, such techniques may result in the loss of certain organochlorine compounds. For this reason great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial wastes.

3.3 Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection. These materials do not interfere when the microcoulometric or electrolytic conductivity detectors are used in the halogen mode.

3.4 Organochlorine pesticides and other halogenated compounds constitute interferences in the determination of PCB's. Most of these are separated by the method described below. However, certain compounds, if present in the sample, will occur with the PCB's. Included are: Sulfur, Heptachlor, aldrin, DDE, technical chlordane, mirex, and to some extent o,p'-DDT and p,p'-DDT.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection part.

4.2 Detector Options:

4.2.1 Electron Capture - Radioactive (tritium or nickel-63)

4.2.2 Microcoulometric Titration

4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with detector system.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom Q (100-120 mesh)

4.4.4 Liquid Phases - Expressed as weight percent coated on solid support:

4.4.4.1 SE-30 or OV-1, 3%

4.4.4.2 OV-17, 1.5% + QF-1, 1.95%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Columns - three ball (macro)

4.5.2 Evaporate Flasks 500 ml

4.5.3 Receiver Ampuls - 10 ml, graduated

4.5.4 Ampul stoppers

4.6 Chromatographic Column - Chromaflex (400 mm long X 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - Pyrex (approximately 400 mm long X 20 mm ID) with a coarse fritted plate on bottom.

4.8 Micro Column Pyrex - constructed according to Figure 1.

4.9 Capillary pipets disposable (5-3/4 in.) with rubber bulb. (Scientific Products P5205-1).

4.10 Low pressure regulator - 0 to 5 PSIG - with low-flow needle valve (See Figure 1, Matheson Model 70).

4.11 Beaker - 100 ml

4.12 Micro syringes - 10, 25, 50 and 100 µl.

4.13 Separatory Funnels 125 ml, 1000 ml, and 2000 ml with Teflon stopcocks

4.14 Graduated Cylinders - 100 ml, 250 ml.

4.15 Blender - High speed, glass or stainless cup.

4.16 Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 in foil-covered glass container. Determine lauric-acid value (See Appendix I).

4.17 Silica gel - Davison code 950-08-08-226 (60/80 mesh).

4.18 Glass Wool - Hexane extracted.

4.19 Centrifuge Tubes - Pyrex calibrated (15 ml).

5. Reagents, Solvents and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.4 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.5 Sodium Sulfate - (ACS) Granular, anhydrous, conditioned for 4 hours @ 400 C.

5.6 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.7 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.7.1 Must contain 2% alcohol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.7.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.7.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.8 n-Hexane - Pesticide quality (NOT MIXED HEXANES).

5.9 Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (Boiling range 30-60 C) - pesticide quality, redistill in glass if necessary.

5.10 Standards - Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016.

5.11 Anti-static Solution - STATNUL, Daystrom, Inc., Weston Instrument Division, Newark, N.J. 95212.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable when the response to dicapthon is at least 50% of full scale when $\leq$ .06 ng is injected for electron capture detection and $\leq$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards are injected frequently as a check on the stability of operating conditions, detector and column. Example chromatograms are shown in Figures 3 through 8 and provide reference operating conditions.

Quality Control

7.1 Duplicate and spiked sample analyses are recommended as a quality control check. When the routine occurrence of a pollution parameter is observed, quality control charts are also recommended (5).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equal to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 For a sensitivity requirement of 1 μg/l, when using microcoulometric or electrolytic conductivity methods for detection take 100 ml of sample for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.

8.3 Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

9. Extraction

9.1 Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 6-10 ml in the K-D evaporator on a hot water bath.

9.4 Qualitatively analyze the sample by gas chromatography with an electron capture detector. From the response obtained decide:

a. If there are any organochlorine pesticides present,

b. If there are any PCB's present,

c. If there is a combination of a and b,

d. If elemental sulfur is present,

e. If the response is too complex to determine a, b, or c.

f. If no response, concentrate to 1.0 ml or less, as required, according to EPA Method (4), pg. 28 and repeat the analysis looking for a, b, c, d, and e. Samples containing Aroclors with a low percentage of chlorine, eg. 1221 and 1232, may require this concentration in order to achieve the detection limit of 1 µg/l. Trace quantities of PCB's are often masked by background which usually occur in the samples.

9.5 If condition a exists, quantitatively determine the organochlorine pesticides according to (1).

9.6 If condition b exists, PCB's only are present, no further separation or cleanup is necessary. Quantitatively determine the PCB's according to 11. below.

9.7 If condition c exists, compare peaks obtained from the sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCB's from the organochlorine pesticides, continue as outlined in 10.4.

9.8 If condition d exists separate the sulfur from the sample using the method outlined in (10.3) followed by the method in (10.5).

9.9 If condition e exists then the following macro cleanup and separation procedures (10.2 and 10.3) should be employed and, if necessary, followed by the micro separation procedures (10.4 and 10.5).

10. Cleanup and Separation Procedures

10.1 Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as, the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether cleanup is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

10.2 Acetonitrile Partition - This procedure is used to remove fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for the compounds of interest.

10.2.1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.

10.2.2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-35 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.

10.2.3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D

flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

10.2.4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

10.2.5 Analyze by gas chromatography unless a need for further cleanup is indicated.

10.3 Florisil Column Adsorption Chromatography

10.3.1 Adjust the sample extract volume to 10 ml.

10.3.2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition below). Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin	DDT	Pentachloro-nitrobenzene
BHC	Heptachlor	
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin
	Mirex	PCB's

15% Eluate	50% Eluate
Endosulfan I	Endosulfan II
Endrin	Captan
Dieldrin	
Dichloran	
Phthalate esters	

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual (6).

10.3.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

10.3.5 Analyze by gas chromatography.

10.4 Silica Gel Micro-Column Separation Procedure (7)

10.4.1 Activation for Silica Gel

10.4.1.1 Place about 20 gm of silica gel in a 100 ml beaker. Activate at 180 C for approximately 16 hours. Transfer the silica gel to a 100 ml glass stoppered bottle. When cool, cover with about 35 ml of 0.50% diethyl ether in benzene (volume:volume). Keep bottle well sealed. If silica gel collects on the ground glass surfaces, wash off with the above solvent

before resealing. Always maintain an excess of the mixed solvent in bottle (approximately 1/2 in above silica gel). Silica gel can be effectively stored in this manner for several days.

10.4.2 Preparation of the Chromatographic Column

10.4.2.1 Pack the lower 2 mm ID Section of the microcolumn with glass wool. Permanently mark the column 120 mm above the glass wool. Using a clean rubber bulb from a disposable pipet seal the lower end of the microcolumn. Fill the microcolumn with 0.50% ether in benzene (v:v) to the bottom of the 10/30 joint (Figure 1). Using a disposable capillary pipet, transfer several aliquots of the silica gel slurry into the microcolumn. After approximately 1 cm of silica gel collects in the bottom of the microcolumn, remove the rubber bulb seal, tap the column to insure that the silica gel settles uniformly. Carefully pack column until the silica gel reaches the 120 ± 2 mm mark. Be sure that there are no air bubbles in the column. Add about 10 mm of sodium sulfate to the top of the silica gel. Under low humidity conditions, the silica gel may coat the sides of the column and not settle properly. This can be minimized by wiping the outside of the column with an anti-static solution.

10.4.2.2 Deactivation of the Silica Gel

a. Fill the microcolumn to the base of the 10/30 joint with the 0.50% ether-benzene mixture, assemble reservoir (using spring clamps) and fill with approximately 15 ml of the 0.50% ether-benzene mixture. Attach the air pressure device (using spring clamps) and adjust the elution rate to approximately 1 ml/min. with the air pressure control. Release the air pressure and detach reservoir just as the last of the solvent enters the sodium sulfate. Fill the column with n-hexane (not mixed hexanes) to the base of the 10/30 fitting. Evaporate all residual benzene from the reservoir, assemble the reservoir section and fill with 5 ml of n-hexane. Apply air pressure and adjust the flow to 1 ml/min. (The n-hexane flows slightly faster than the benzene). Release the air pressure and remove the reservoir just as the n-hexane enters the sodium sulfate. The column is now ready for use.

b. Pipet a 1.0 ml aliquot of the concentrated sample extract (previously reduced to a total volume of 2.0 ml) on to the column.

As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with 0.25 ml of n-hexane. Then assemble the upper section of the column. As the last of the n-hexane rinse reaches the surface of the sodium sulfate, add enough n-hexane (volume predetermined, see 10.4.3 below) to just elute all of the PCB's present in the sample. Apply air pressure and adjust until the flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see 10.4.3 below) in an accurately calibrated ampul. As the last of the n-hexane reaches the surface of the sodium sulfate, release the air pressure and change the collection ampul.

c. Fill the column with 0.50% diethyl ether in benzene, again apply air pressure and adjust flow to 1 ml/min. Collect the eluate until all of the organochlorine pesticides of interest have been eluted (volume predetermined, see 10.4.3 below).

d. Analyze the eluates by gas chromatography.

10.4.3 Determination of Elution Volumes

10.4.3.1 The elution volumes for the PCB's and the pesticides depend upon a number of factors which

are difficult to control. These include variation in:

a. Mesh size of the silica gel

b. Adsorption properties of the silica gel

c. Polar contaminants present in the eluting solvent

d. Polar materials present in the sample and sample solvent

e. The dimensions of the microcolumns

Therefore, the optimum elution volume must be experimentally determined each time a factor is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and serially collect 1 ml elution volumes. Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for ether-benzene. Figure 2 shows the retention order of the various PCB components and of the pesticides. Using this information, prepare the mixtures required for calibration of the microcolumn.

10.4.3.2 In determining the volume of hexane required to elute the PCB's the sample volume (1 ml) and the volume of n-hexane used to rinse the column wall must be considered. Thus, if it is determined that a 10.0 ml elution volume is required to elute the PCB's, the volume of hexane to be added

in addition to the sample volume but including the rinse volume should be 9.5 ml.

10.4.3.3 Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (9.4) indicates which Aroclors are present and provides the basis for selection of the ideal elution volume. This helps to minimize the quantity of organochlorine pesticides which will elute along with the low percent chlorine PCB's and insures the most efficient separations possible for accurate analysis.

10.4.3.4 For critical analysis where the PCB's and pesticides are not separated completely, the column should be accurately calibrated according to (10.4.3.1) to determine the percent of material of interest that elutes in each fraction. Then flush the column with an additional 15 ml of 0.50% ether in benzene followed by 5 ml of n-hexane and use this reconditioned column for the sample separation. Using this technique one can accurately predict the amount (%) of materials in each micro column fraction.

10.5 Micro Column Separation of Sulfur, PCB's, and Pesticides

10.5.1 See procedure for preparation and packing micro column in PCB analysis section (10.4.1 and 10.4.2).

10.5.2 Microcolumn Calibration

10.5.2.1 Calibrate the microcolumn for sulfur and PCB separation by collecting 1.0 ml fractions and analyzing them by gas chromatography to determine the following:

1) The fraction with the first eluting PCB's (those present in 1260),
2) The fraction with the last eluting PCB's (those present in 1221),
3) The elution volume for sulfur,
4) The elution volume for the pesticides of interest in the 0.50% ether-benzene fraction.

From these data determine the following:

1) The eluting volume containing only sulfur (Fraction I),
2) The eluting volume containing the last of the sulfur and the early eluting PCB's (Fraction II),
3) The eluting volume containing the remaining PCB's (Fraction III),
4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV).

10.5.3 Separation Procedure

10.5.3.1 Carefully concentrate the 6% eluate from the florisil column to 2.0 ml in the graduated ampul on a warm water bath.

10.5.3.2 Place 1.0 ml (50%) of the concentrate into the microcolumn with a 1 ml pipet. Be careful

not to get any sulfur crystals into the pipet.

10.5.3.3 Collect Fractions I and II in calibrated centri fuge tubes.

Collect Fractions III and IV in calibrated ground glass stoppered ampules.

10.5.3.4 Sulfur Removal (9) - Add 1 to 2 drops of mercury to Fraction II stopper and place on a wrist-action shaker. A black precipitate indicates the presence of sulfur. After approxiately 20 minutes the mercury may become entirely reacted or deactivated by the precipitate. The sample should be quantitatively transferred to a clean centrifuge tube and additional mercury added. When crystals are present in the sample, three treatments may be necessary to remove all the sulfur. After all the sulfur has been removed from Fraction II (check using gas chromatography) combine Fractions II and III. Adjust the volume to 10 ml and analyze gas chromatography. Be sure no mercury is transferred to the combined Fractions II and III, since it can react with certain pesticides.

By combining Fractions II and III, if PCB's are present, it is possible to identify the Aroclor(s) present and a quantitative analysis can be performed accordingly. Fraction I can be discarded since it only contains the bulk of the sulfur. Analyze Fractions III and IV for the PCB's and

pesticides. If DDT and its homologs, aldrin, heptachlor, or technical chlordane are present along with the PCB's, an additional micro-column separation can be performed which may help to further separate the PCB's from the pesticides (See 10.4).

11. Quantitative Determination

11.1 Measure the volume of n-hexane eluate, containing the PCB's and inject 1 to 5 µl into the gas chromatograph. If necessary, adjust the volume of the eluate to give linear response to the electron capture detector. The microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCB's.

11.2 Calculations

11.2.1 When a single Aroclor is present, compare quantitative Aroclor reference standards (e.g., 1242, 1260) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Microgram/liter} = \frac{[A]\ [B]\ [V_t]}{[(V_i)\ (V_s)]} \times [N]$$

$$A = \frac{\text{ng of Standard Injected}}{\Sigma \text{ of Standard Peak Areas}} = \frac{ng}{mm^2}$$

$B = \Sigma$ of Sample Peak Areas = (mm^2)

V_i = Volume of sample injected (µl)

V_t = Volume of Extract (µl) from which sample is injected into gas chromatograph

V_s = Volume of water sample extracted (ml)

N = 2 when micro column used
1 when micro column not used

Peak Area = Peak height (mm x Peak Width at 1/2 height

11.2.2 For complex situations, use the calibration method described below. Small variations in components between different Aroclor batches make it necessary to obtain samples of several specific Aroclors. These reference Aroclors can be obtained from Dr. Ronald Webb, Southest Environmental Research Laboratory, EPA, Athens, Georgia 30601. The procedure is as follows:

11.2.2.1 Using the OV-1 column, chromatograph a known quantity of each Aroclor reference standard. Also chromatograph a sample of p,p'-DDE. Suggested concentration of each standard is 0.1 ng/µl for the Aroclors and 0.02 ng/µl for the p,p'-DDE.

11.2.2.2 Determine the relative retention time (RRT) of each PCB peak in the resulting chromatograms using p,p'-DDE as 100. See Figures 3 through 5

$$RRT = \frac{RT \times 100}{RT_{DDE}}$$

RRT = Relative Retention Time

RT = Retention time of peak of interest

RT_{DDE} = Retention time of p,p'-DDE

Retention time is measured as that distance in mm between the first appearance of the solvent peak and the maximum for the compound.

11.2.2.3 To calibrate the instrument for each PCB measure the area of each peak.

Area = Peak height (mm) x Peak width at 1/2 height. Using Tables 1 through 6 obtain the proper mean weight factor, then determine the response factor ng/mm^2.

$$ng/mm^2 = \frac{(ng_i)\ \frac{(\text{mean weight percent})}{100}}{(\text{Area})}$$

ng_i = ng of Aroclor Standard Injected

Mean weight percent = obtained from Tables 1 through 6.

11.2.2.4 Calculate the RRT value and the area for each PCB peak in the sample chromatogram. Compare the sample chromatogram to those obtained for each reference Aroclor standard. If it is apparent that the PCB peaks present are due to only one Aroclor then calculate the concentration of each PCB using the following formula:

ng PCB = ng/mm^2 x Area

Where Area = Area (mm^2) of sample peak

ng/mm^2 = Response factor for that peak measured.

Then add the nanograms of PCB's present in the injection to get the total number of nanograms of PCB's present. Use the following formula to calculate the concentration of PCB's in the sample:

$$\text{Micrograms/Liter} = \frac{[ng]\ \ [V_t]}{[V_s]\ \ [V_i]} \times [N]$$

V_s = volume of water extracted (ml)

V_t = volume of extract (µl)

V_i = volume of sample injected (µl)

Σng = sum of all the PCB's in nanograms for that Aroclor identified

N = 2 when microcolumn used

N = 1 when microcolumn not used

The value can then be reported as Micrograms/Liter PCB's reported as the Aroclor. For samples containing more than one Aroclor, use Figure 9 chromatogram divisional flow chart to assign a proper response factor to each peak and also identify the "most likely" Aroclors present. Calculate the ng of each PCB isomer present and sum them according to the divisional flow chart. Using the formula above, calculate the concentration of the various Aroclors present in the sample.

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

Table 1

Composition of Aroclor 1221 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	31.8	15.8	1
14	19.3	9.1	1
16	10.1	9.7	2
19	2.8	9.7	2
21	20.8	9.3	2
28	5.4	13.9	2] 85% 3] 15%
32	1.4	30.1	2] 10% 3] 90%
[37 [40	1.7	48.8	3 3
Total	93.3		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of seventeen results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 2

Composition of Aroclor 1232 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	16.2	3.4	1
14	9.9	2.5	1
16	7.1	6.8	2
[20	17.8	2.4	2
[21			2
28	9.6	3.4	2] 40%
			3] 60%
32	3.9	4.7	3
37	6.8	2.5	3
40	6.4	2.7	3
47	4.2	4.1	4
54	3.4	3.4	3] 33%
			4] 67%
58	2.6	3.7	4
70	4.6	3.1	4] 90%
			5] 10%
78	1.7	7.5	4
Total	94.2		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of four results as a mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 3

Composition of Aroclor 1242 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
11	1.1	35.7	1
16	2.9	4.2	2
21	11.3	3.0	2
28	11.0	5.0	2] 25% 3] 75%
32	6.1	4.7	3
37	11.5	5.7	3
40	11.1	6.2	3
47	8.8	4.3	4
54	6.8	2.9	3] 33% 4] 67%
58	5.6	3.3	4
70	10.3	2.8	4] 90% 5] 10%
78	3.6	4.2	4
84	2.7	9.7	5
98	1.5	9.4	5
104	2.3	16.4	5
125	1.6	20.4	5] 85% 6] 15%
146	1.0	19.9	5] 75% 6] 25%
Total	98.5		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 4

Composition of Aroclor 1248 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
21	1.2	23.9	2
28	5.2	3.3	3
32	3.2	3.8	3
47	8.3	3.6	3
40	8.3	3.9	3] 85% 4] 15%
47	15.6	1.1	4
54	9.7	6.0	3] 10% 4] 90%
58	9.3	5.8	4
70	19.0	1.4	4] 80% 5] 20%
78	6.6	2.7	4
84	4.9	2.6	5
98	3.2	3.2	5
104	3.3	3.6	4] 10% 5] 90%
112	1.2	6.6	5
125	2.6	5.9	5] 90% 6] 10%
146	1.5	10.0	5] 85% 6] 15%
Total	103.1		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 5

Composition of Aroclor 1254 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
47	6.2	3.7	4
54	2.9	2.6	4
58	1.4	2.8	4
70	13.2	2.7	4] 25%
			5] 75%
84	17.3	1.9	5
98	7.5	5.3	5
104	13.6	3.8	5
125	15.0	2.4	5] 70%
			6] 30%
146	10.4	2.7	5] 30%
			6] 70%
160	1.3	8.4	6
174	8.4	5.5	6
203	1.8	18.6	6
232	1.0	26.1	7
Total	100.0		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent.

[b]Standard deviation of six results as a percentage of the mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers are bracketed.

Table 6

Composition of Aroclor 1260 (8)

RRT[a]	Mean Weight Percent	Relative Std. Dev.[b]	Number of Chlorines[c]
70	2.7	6.3	5
84	4.7	1.6	5
[98	3.8	3.5	] [d]
104]			5] 60%
			6] 40%
117	3.3	6.7	6
125	12.3	3.3	5] 15%
			6] 85%
146	14.1	3.6	6
160	4.9	2.2	6] 50%
			7] 50%
174	12.4	2.7	6
203	9.3	4.0	6] 10%
			7] 90%
[232			] [e]
244]	9.8	3.4	6] 10%
			7] 90%
280	11.0	2.4	7
332	4.2	5.0	7
372	4.0	8.6	8
448	.6	25.3	8
528	1.5	10.2	8
Total	98.6		

[a]Retention time relative to p,p'-DDE=100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

[b]Standard deviation of six results as a mean of the results.

[c]From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

[d]Composition determined at the center of peak 104.

[e]Composition determined at the center of peak 232.

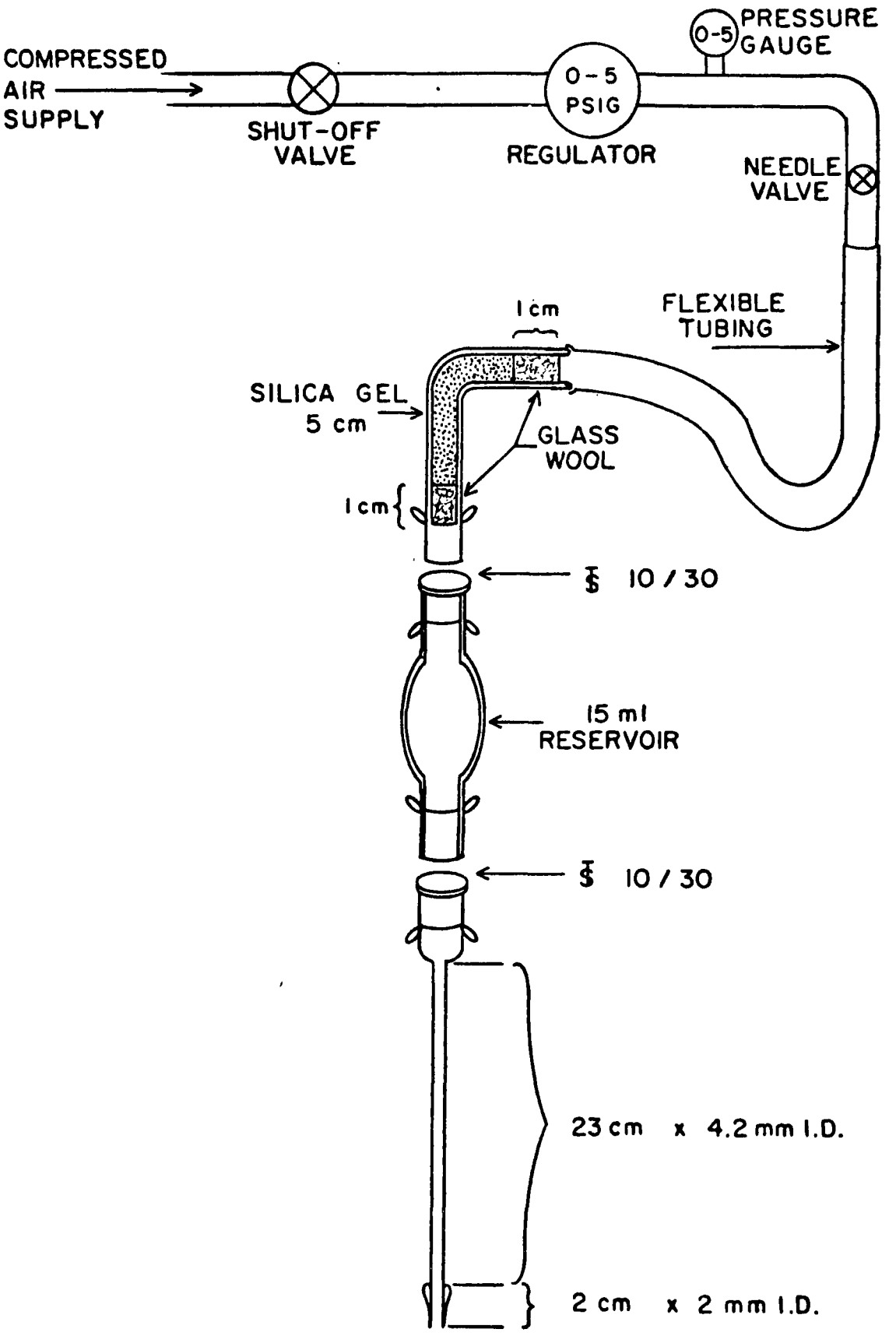


FIGURE 1. MICROCOLUMN SYSTEM

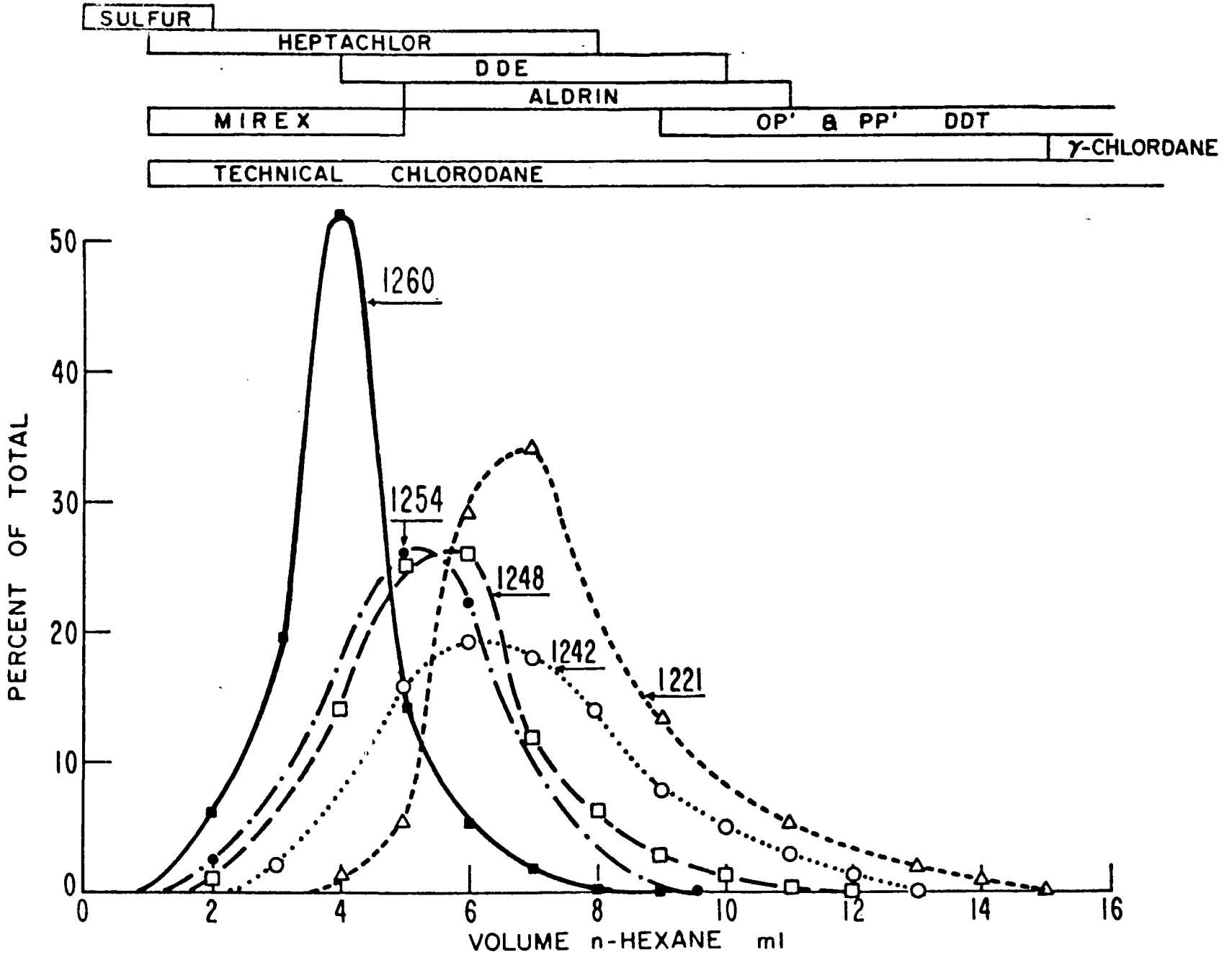


Figure 2. Aroclor Elution Patterns

REFERENCES

(1) "Method for Organochlorine Pesticides in Industrial Effluents," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(2) Leoni, V., "The Separation of Fifty Pesticides and Related Compounds and Polychlorinated Biphenyls into Four Groups by Silica Gel Microcolumn Chromatography," Journal of Chromatography, 62, 63 (1971).

(3) McClure, V. E., "Precisely Deactivated Adsorbents Applied to the Separation of Chlorinated Hydrocarbons," Journal of Chromatography, 70, 168 (1972).

(4) "Methods for Organic Pesticides in Water and Wastewater," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(5) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(6) "Pesticide Analytical Manual," U.S. Dept. of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.

(7) Bellar, T. A. and Lichtenberg, J. J., "Method for the Determination of Polychlorinated Biphenyls in Water and Sediment," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1973.

(8) Webb, R. G. and McCall, A. C., "Quantitative PCB Standards for Electron Capture Gas Chromatography." Presented at the 164th National ACS Meeting, New York, August 29, 1972. (Submitted to the Journal of Chromatographic Science for publication).

(9) Goerlitz, D. F. and Law, L. M., "Note on Removal of Sulfur Interferences from Sediment Extracts for Pesticide Analysis," Bulletin of Environmental Contamination and Toxicology, 6, 9 (1971).

(10) Mills, P. A., "Variation of Florisil Activity: Sample Method for Measuring Adsorbent Capacity and its Use in Standardizing Florisil Columns," Journal of the Association of Official Analytical Chemists, 51, 29 (1968).

(11) Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

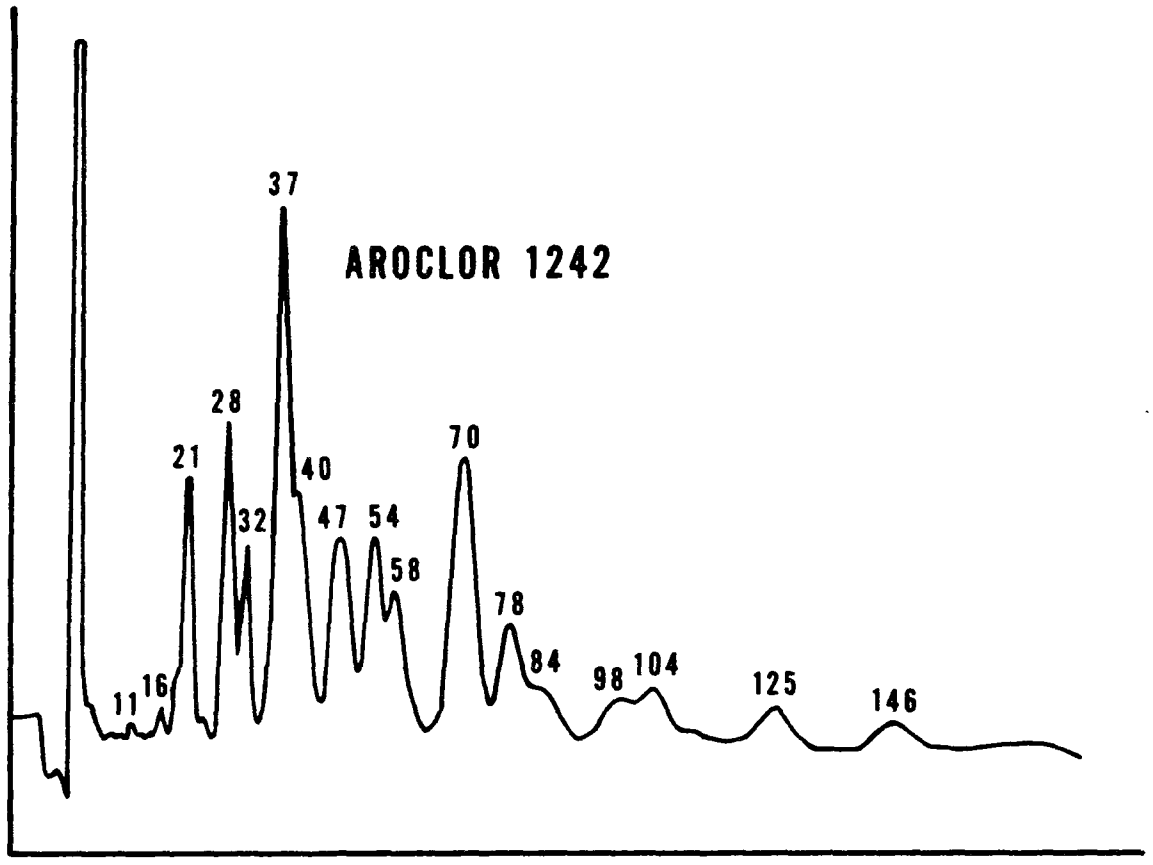


Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture

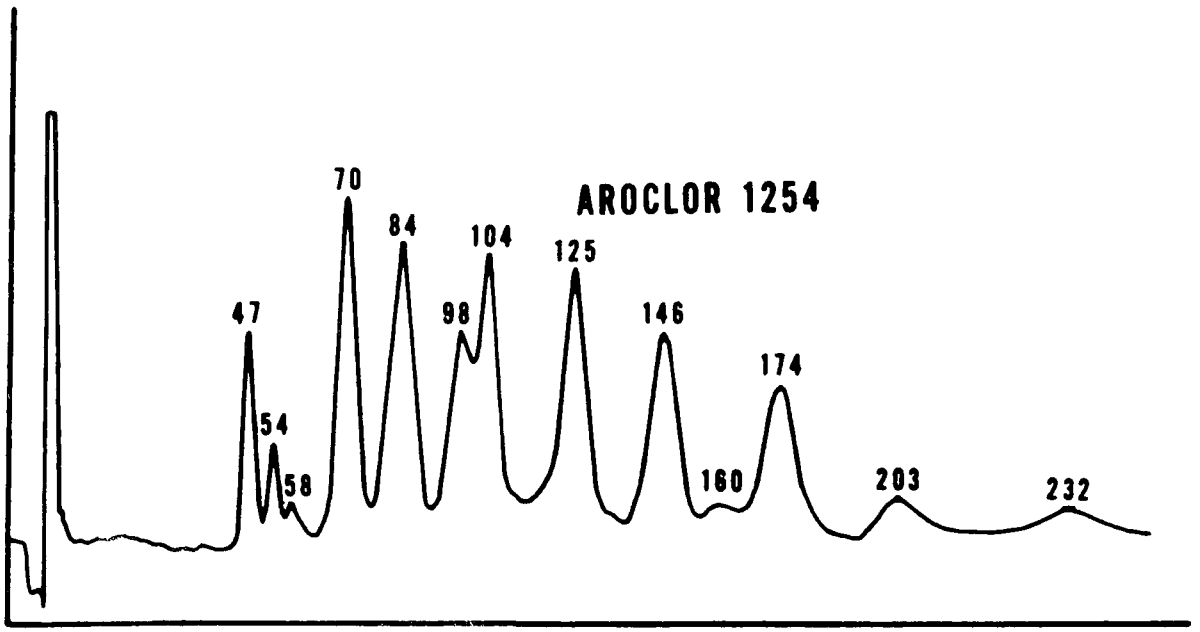


Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

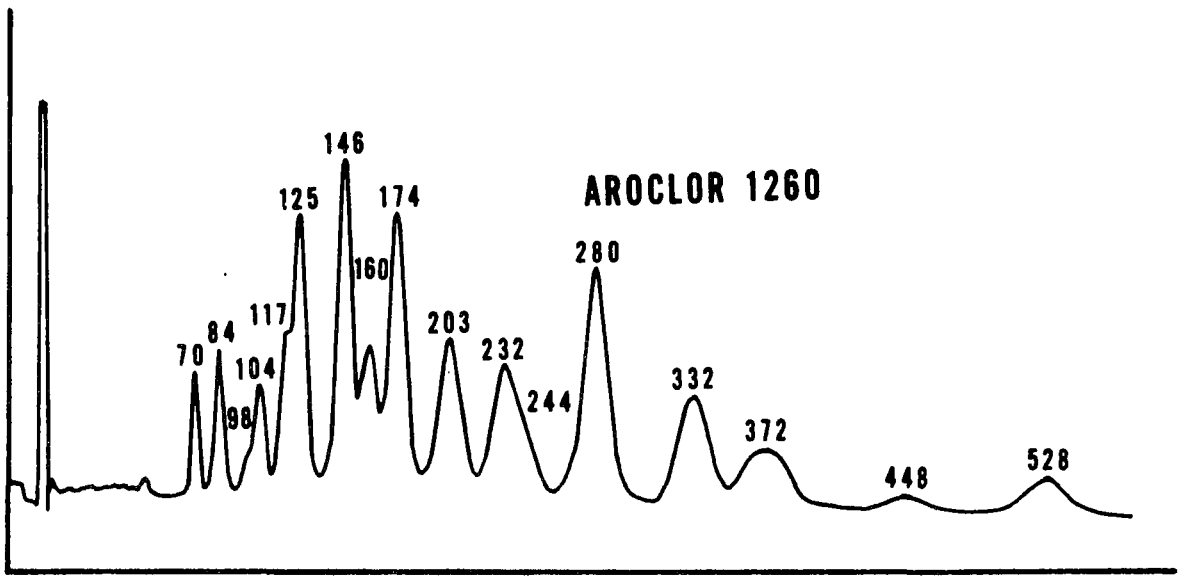


Figure 5. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 170 C, Detector: Electron Capture.

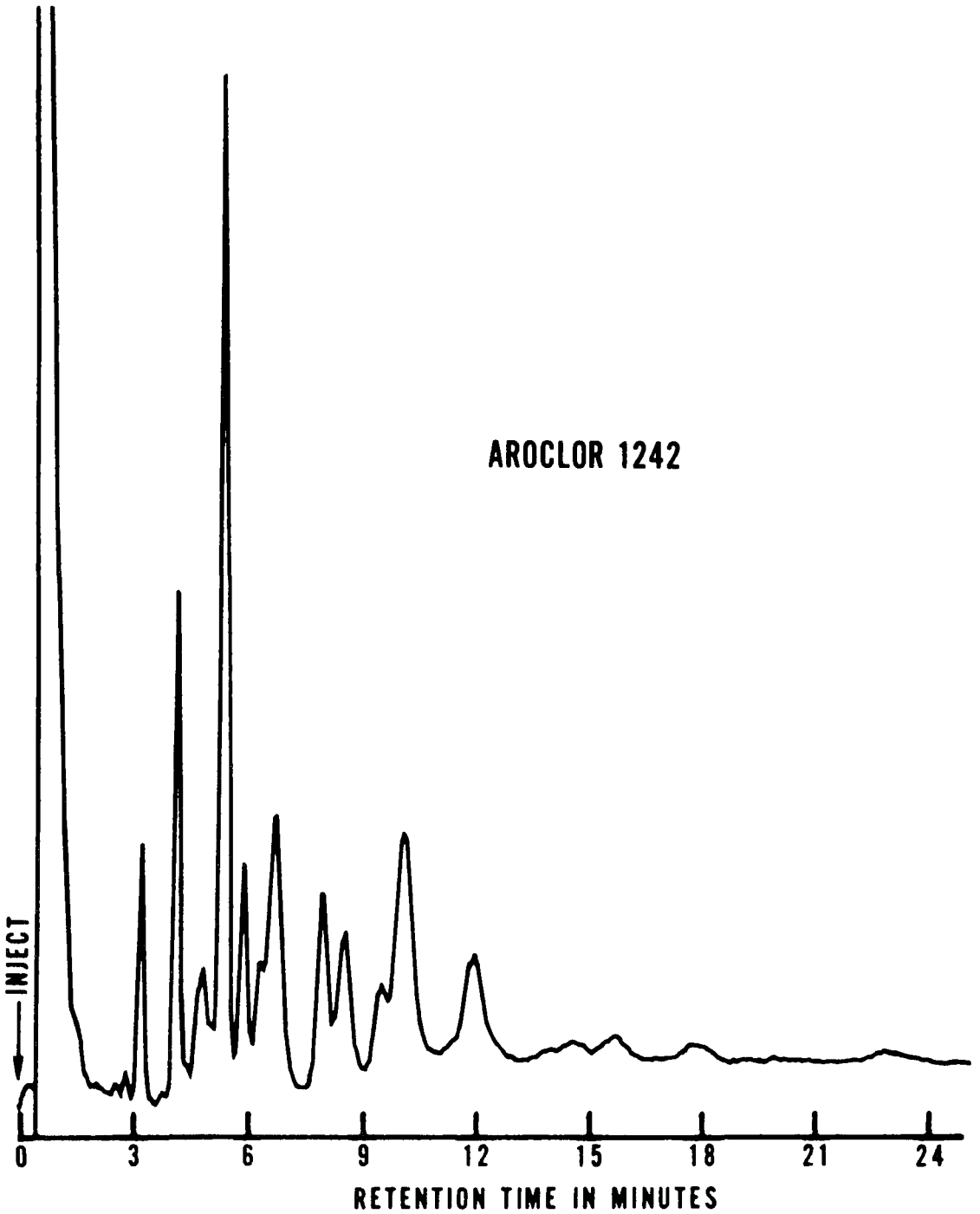


Figure 6. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture

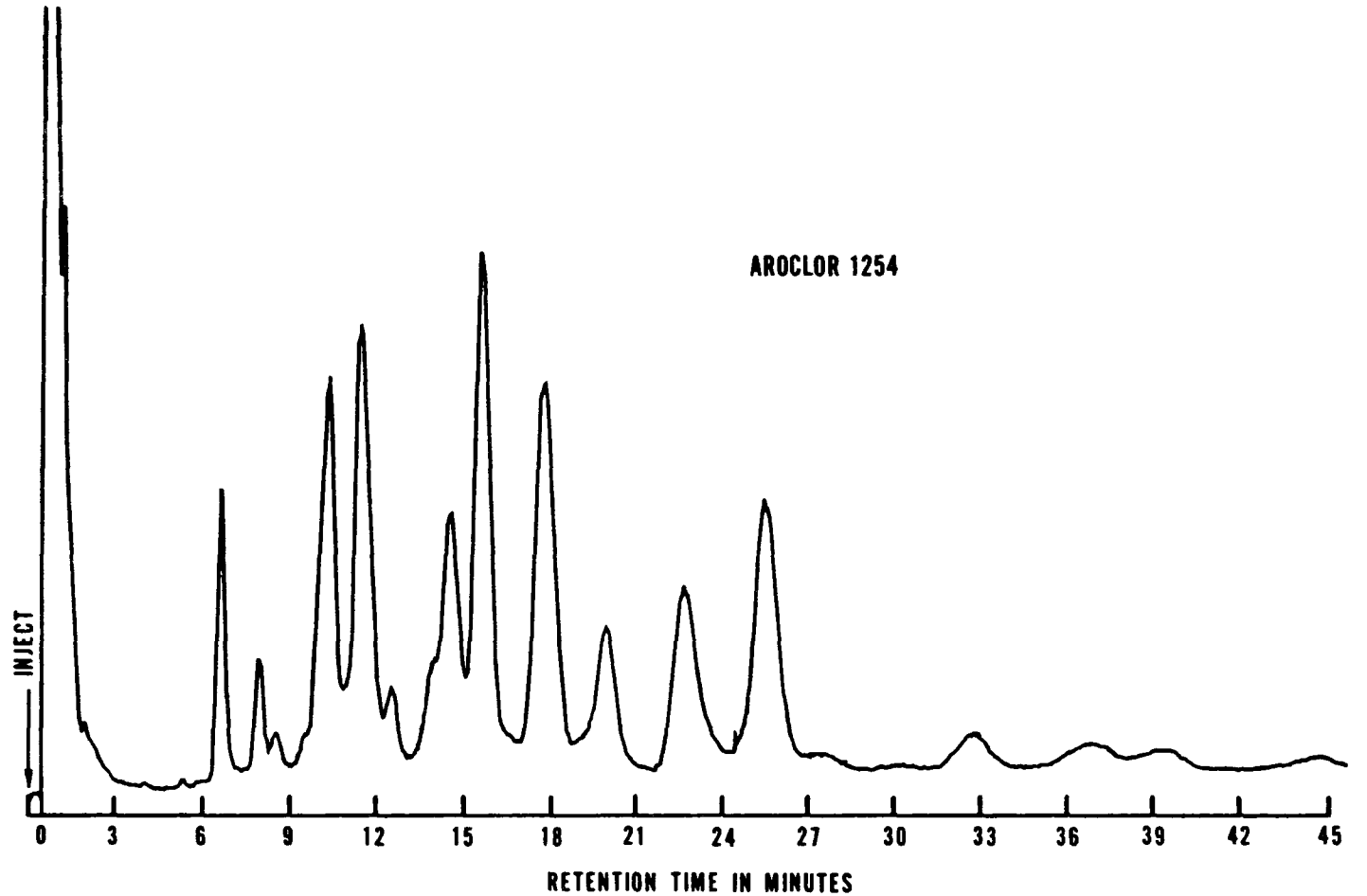


Figure 7. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

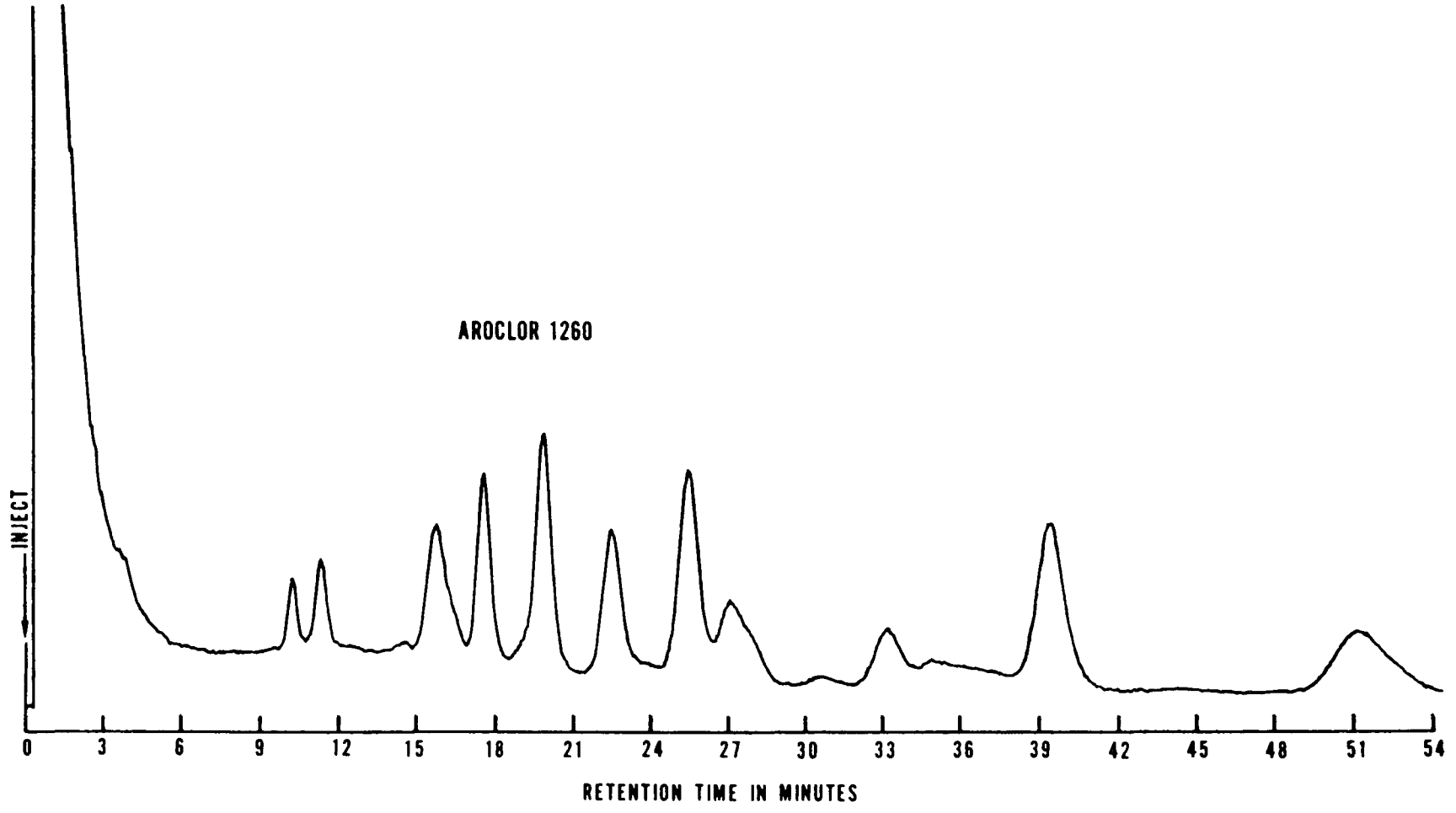


Figure 8. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200C, Detector: Electron Capture.

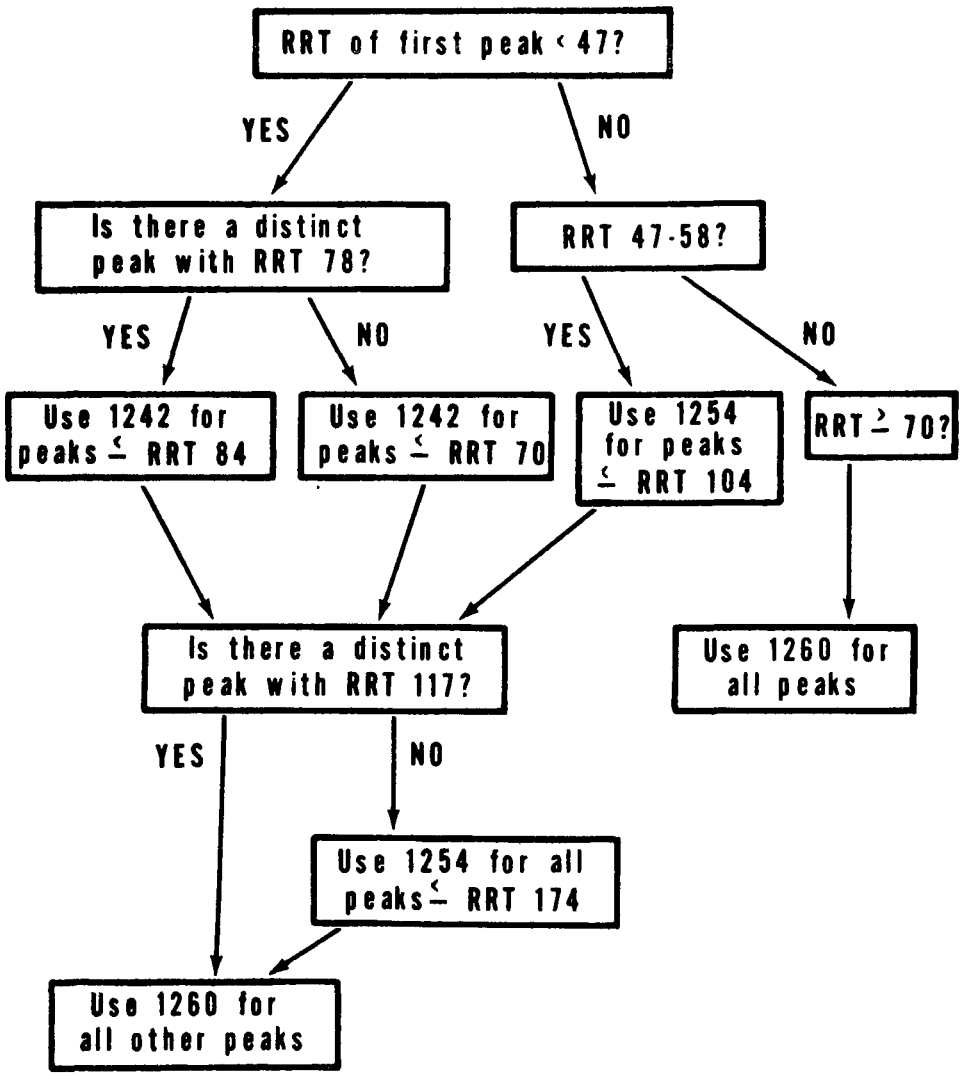


Figure 9. Chromatogram Division Flowchart (8).

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 758-486/1021

[Fed. Reg., 38, No. 75, Pt. II]

4. METHOD FOR TRIAZINE PESTICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of various symmetrical triazine pesticides.

1.2 The following compounds may be determined by this method with a sensitivity of 1 µg/1: ametryne, atratone, atrazine, GS-13529, GS-14254, prometone, prometryne, propazine, and simazine. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

1.3 Individual triazines may be difficult to identify and quantitate in the presence of other triazines or other nitrogen-containing compounds. Provisions incorporated in this method are intended to minimize the effect of such interferences.

2. Summary

2.1 The method describes an efficient sample extraction procedure and provides, through use of column chromatography, a method for the elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separation, and measurement is accomplished by the use of an electrolytic conductivity detector (CCD). Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing

misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to (1) Part 1, Sections 1.4 and 1.5.

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of triazine pesticides. The use of a specific detector supported by an optional column cleanup procedure will eliminate many of these interferences.

3.3 Nitrogen containing compounds other than the triazines may interfere.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass-lined injection port.

4.2 Detector - Electrolytic Conductivity.

4.3 Recorder - Potentiometric strip chart (10 in) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom Q (100-120 mesh)

4.4.4 Liquid Phase - Expressed as weight percent coated on solid support

4.4.4.1 Carbowax 20M, 1%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (K-503000)

4.5.2 Micro-Snyder Column - two ball (K-569001)

4.5.3 Evaporative Flasks - 500 ml (K-570001)

4.5.4 Receiver Ampuls - 10 ml, graduated (K-570050)

4.5.5 Ampul Stoppers

4.6 Chromatographic Column - Chromaflex (400 mm long X 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - Pyrex (approximately 400 mm long X 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50, and 100 µl.

4.9 Separatory Funnels - 2000 ml with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Graduated Cylinders - 1000 ml.

4.12 Florisil - PR Grade (60-80 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

5. Reagents, Solvents and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.4 Sodium Sulfate - (ACS) Granular, anhydrous.

5.5 Sulfuric Acid (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.6 Diethyl Ether - Pesticide Quality, redistilled in glass, if necessary

5.6.1 Must contain 2% alcohol and be free of peroxides by the following test: To 10 ml of ether in glass-stoppered

cylinder previously rinsed with ether, add 1 ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.6.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.6.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.7 Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - pesticide quality, redistill in glass if necessary.

5.8 Pesticide Standrads - Reference grade.

6. Calibration

6.1 Gas chromatographic operating conditions are considered optimum when an injection of $\leq$ 20 ng of each triazine will yield a peak at least 50% of full scale deflection with the modified Coulson detector (2). For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Inject standards frequently as a check on the stability of operating conditions. A chromatogram of a mixture of several pesticides is shown in Figure 1 and provides reference operating conditions for the recommended column.

6.3 The elution order and retention ratios of various triazine pesticides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended (3)

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 Quantitatively transfer a 1000 ml aliquot into a two-liter separatory funnel.

9. Extraction

9.1 Add 60 ml methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent, add the solvent to the separatory funnel, and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by gas chromatography.

10. Florisil Column Cleanup

10.1 Adjust the sample extract to 10 ml with hexane.

10.2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

10.4 Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

15% Eluate	50% Eluate	100% Eluate
Propazine (90%)	Propazine (10%)	Atratone
GS-13529 (30%)	GS-13529 (70%)	GS-14254
Atrazine (20%)	Atrazine (80%)	Prometone
	Ametryne	
	Prometryne	
	Simazine	

10.5 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 ml.

10.6 Analyze by gas chromatography.

11. Calculation of Results

11.1 Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2(4)

$$\text{Micrograms/liter} = \frac{(A)\ (B)\ (V_t)}{(V_i)\ \ (V_s)}$$

A = $\frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

V_s = Volume of water extracted (ml)

12. Reporting Results

12.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

TABLE 1

RETENTION RATIOS OF VARIOUS TRIAZINE PESTICIDES RELATIVE TO ATRAZINE

Pesticide	Retention Ratio
Prometone	0.52
Atratone	0.67
Propazine	0.71
GS-13529	0.78
GS-14254	0.88
Atrazine	1.00
Prometryne	1.10
Simazine	1.35
Ametryne	1.48

Absolute retention time of atrazine = 10.1 minutes

REFERENCES:

(1) "Methods for Organic Pesticides in Water and Wastewater", U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(2) Patchett, G. G., "Evaluation of the Electrolytic Conductivity Detector for Residue Analyses of Nitrogen-Containing Pesticides", Journal of Chromatographic Science, 8, 155 (1970).

(3) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, Section 6.4, U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(4) Cochrane, W. P. and Wilson, B. P., "Electrolytic conductivity detection of some nitrogen-containing herbicides", Journal of Chromatography, 63, 364 (1971).

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

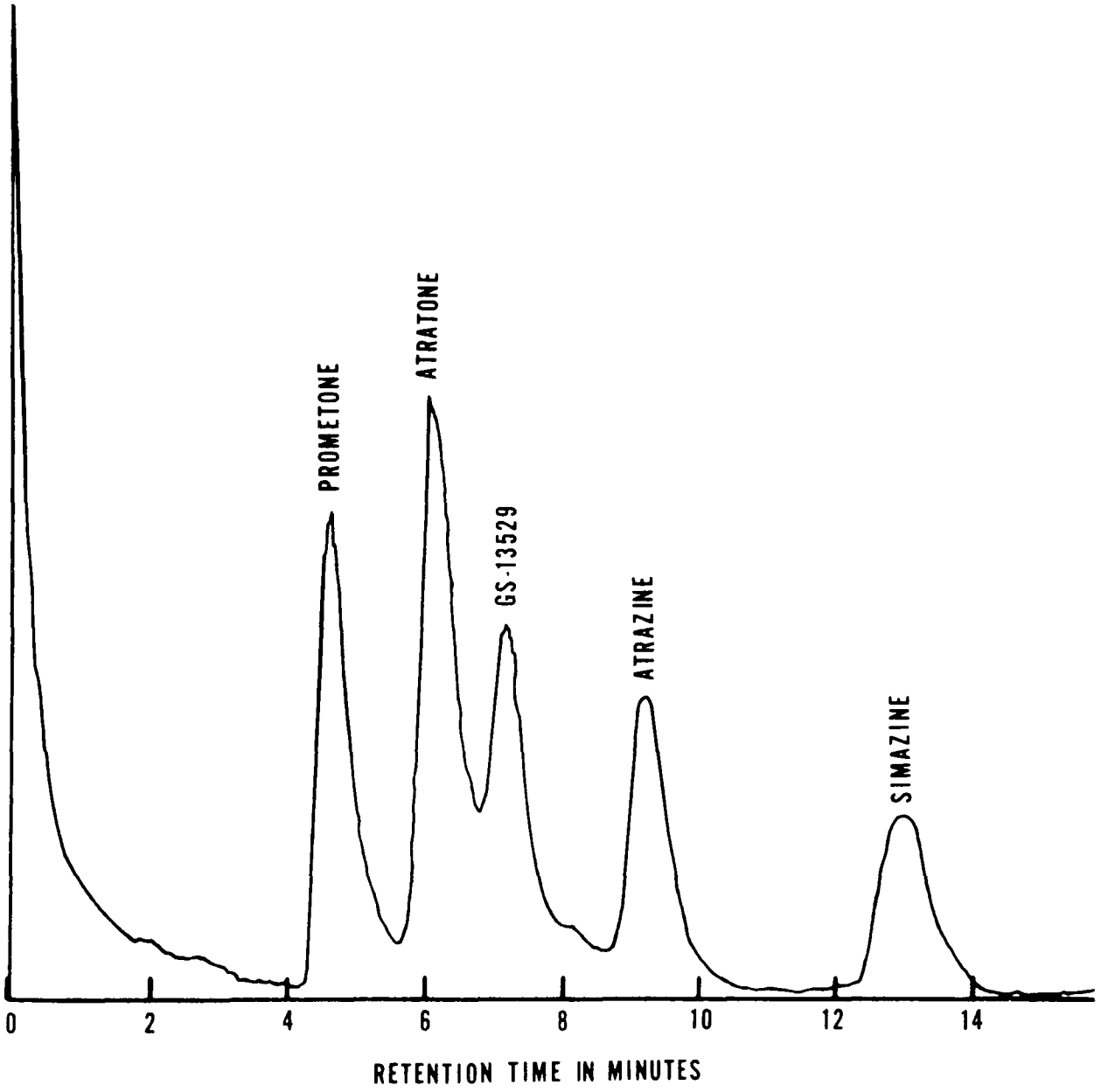


Figure 1. Column Packing: 1% Carbowax 20M on Gas-Chrom Q (100/120 mesh), Column Temperature : 155 C, Carrier Gas: Helium at 80 ml/min, Detector: Electrolytic Conductivity.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1148

5. METHOD FOR O-ARYL CARBAMATE PESTICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of various O-aryl carbamate pesticides in industrial effluents. Such compounds are characterized by the carbamate structure with the oxygen atom attached to an aromatic ring.

1.2 The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: Baygon, carbaryl (Sevin), Matacil, Mesurol, and Zectran. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

1.3 The method also detects phenols and can be extended to the detection of phenolic hydrolysis products of the compounds above.

2. Summary

2.1 A measured volume of water is extracted with methylene chloride. The concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer chromatography. The carbamates are hydrolyzed on the layer and the hydrolysis products are reacted with 2,6-dibromoquinone chlorimide to yield specific colored products. Quantitative measurement is achieved by visually comparing the responses of sample extracts to the responses of standards on the same thin-layer. Identifications are confirmed by changing the pH of the layer and observing color changes of the reaction products. Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

Fed. Reg., 38, No. 75, Pt. II

3. Interferences

3.1 Direct interferences may be encountered from phenols that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the carbamates. In cases where phenols are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the carbamates.

3.2 Indirect interferences may be encountered from naturally colored materials whose presence masks the carbamate reaction.

4. Apparatus and Materials

4.1 Thin layer plates - Glass plates (200 X 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder)

4.2 Spotting template

4.3 Developing chamber

4.4 Sprayer - 20 ml capacity

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - Three ball (K-503000)

4.5.2 Micro-Snyder Column - Two ball (K-569001)

4.5.3 Evaporative Flasks - 500 ml (K-570001)

4.5.4 Receiver Ampuls - 10 ml graduated (K-570050)

4.5.5 Ampul Stoppers

4.6 Chromatographic Column - Chromaflex (400 mm long X 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - Pyrex (approximately 400 mm long X 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50 and 100 µl.

4.9 Separatory Funnel - 2000 ml, with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Florisil PR Grade (60-80 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

5. Reagents, Solvents and Standards

5.1 Ferrous Sulfate - (ACS) 3% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.4 Sodium Sulfate - (ACS) Granular, anhydrous.

5.5 Sulfuric Acid (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.6 Diethyl Ether Nanograde, redistilled in glass, if necessary.

5.6.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.6.2 Decompose ether peroxides by adding 40g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.6.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.7 Hexane, Methanol, Methylene Chloride, Petroleum Ether - Nanograde, redistill in glass if necessary.

5.8 Pesticide Standards - Reference grade.

5.8.1 TLC standards - 0.100 µg/µl in chloroform.

5.9 Chromogenic agent - Dissolve 0.2 g 2,6-dibromoquinone chlorimide in 20 ml chloroform.

5.10 Buffer solution - 0.1 N sodium borate in water .

6. Calibration

6.1 To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

6.2 Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

6.3 For qualitative and quantitative work, spot a series of standards representing 0.1 - 1.0 µg of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

9. Extraction

9.1 Add 60 ml methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a chromatographic column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent, add the solvent to the separatory funnel, and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Synder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section 11).

10. Florisil Column Cleanup

10.1 Adjust the sample extract to 10 ml with hexane.

10.2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether in petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

50% Eluate	100% Eluate
Sevin (70%)	Sevin (30%)
Zectran	Baygon
	Matacil

10.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5 - 1.0 ml.

10.5 Analyze according to 11. below.

11. Separation and Detection

11.1 Carefully spot 10% of the extract on a thin-layer. On the same plate spot several pesticides or mixtures for screening purposes or a series of 1,2,4,6,8 and 10 µl of specific standards for quantitative analysis.

11.2 Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.

11.3 Spray the layer rapidly and evenly with about 10-15 ml chromogenic reagent. Heat the layer in an oven at 110 C for 15 minutes. The pesticides will appear with colors as indicated in Table 2. Make quantitative estimates by visually comparing the intensity and size of the spots with those of the series of standard.

11.4 Spray the layer with sodium borate reagent and observe the color shift of the reaction products. The color shift must be the same for sample and standard for identification to be confirmed.

12. Calculation of Results

12.1 Determine the concentration of pesticide in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter.

13. Reporting Results

13.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

REFERENCES CITED:

(1) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, Section 6.4, U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(2) "Methods for Organic Pesticides in Water and Wastewater", U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(3) Finocchiaro, J. M. and Benson, W. R., "Thin-Layer Chromatography of Some Carbamate and Phenyl Urea Pesticides", Journal of the Association of Official Agricultural Chemist, 50, 888 (1967).

(4) Smith, D. and Lichtenberg, J. J., "Determination of Phenols in Surface Waters by Thin-Layer Chromatography", Microorganic Matter in Water, ASTM STP 448, American Society for Testing and Materials, 1969, pp. 78-95.

(5) Stahl, E., "Thin-Layer Chromatography", Academic Press, New York, 1969

(6) Longbottom, J. E. and Lichtenberg, J. J., "Determination of Carbamate and Urea Pesticides in Surface Waters by Thin-Layer Chromatography", U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(7) Steere, N. V., editor, "Handbook of Laboratory Safety", Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

Table 1

R_f Values of O-Aryl Carbamates Pesticides in Several Solvent Systems

	A	B	C	D	E	F
Sevin	0.26	0.22	0.48	0.41	0.58	0.24
Matacil	0.26	0.02	0.46	0.52	0.54	0.04
Zectran	0.34	0.22	0.54	0.53	0.60	0.24
Mesurol	0.31	0.31	0.55	0.55	0.59	0.28
Baygon	0.27	0.10	0.53	0.59	0.60	0.13

Solvent Systems

A. Hexane/acetone (3:1)

B. Methylene chloride

C. Benzene/acetone (4:1)

D. Benzene/cyclohexane/diethylamine (5:2:2)

D. Ethyl acetate

F. Chloroform

TABLE 2

COLOR RESPONSES AND DETECTION LIMIT FOR O-ARYL CARBAMATES

	Colors		Detection Limit (µg)
	Before Buffer	After Buffer	
Sevin	Brown	Red-Purple	0.1
Matacil	Gray	Green	0.1
Zectran	Gray	Green	0.1
Mesural	Brown	Tan	0.2
Baygon	Blue	Blue	0.1

APPENDIX I

13. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1149

6. METHOD FOR N-ARYL CARBAMATE AND UREA PESTICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of various N-aryl carbamate and urea pesticides in industrial effluents. Such compounds are characterized by the carbamate and urea structures with a nitrogen atom attached to an aromatic ring.

1.2 The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: barban, chloropropham, diuron, fenuron, linuron, monuron, neburon, propham, biduron, Swep, Urab and Urox. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.

1.3 The method also detects anilines and can be extended to the detection of anilinic hydrolysis products of the compounds above.

2. Summary

2.1 A measured volume of water is extracted with methylene chloride and the concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer chromatography. The pesticides are hydrolyzed to primary amines, which in turn are chemically converted to diazonium salts. The layer is sprayed with 1-naphthol and the products appear as colored spots. Quantitative measurement is achieved by visually comparing the responses of sample extracts to the responses of standards on the same thin layer. Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Direct interferences may be encountered from aromatic amines that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the pesticides. In cases where amines are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the pesticides on the layer.

3.2 Indirect interferences may be encountered from naturally colored materials whose presence masks the chromogenic reaction.

4. Apparatus and Materials

4.1 Thin-layer plates - Glass plates (200 X 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder).

4.2 Spotting Template

4.3 Developing Chamber

4.4 Sprayer - 20 ml capacity

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (K-503000)

4.5.2 Micro-Snyder Column - two ball (K-569001)

4.5.3 Evaporative Flasks - 500 ml (K-570001)

4.5.4 Receiver Amputs - 10 ml graduated (K-570050)

4.6 Chromatographic Column - Chromaflex (400 mm long X 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

4.7 Chromatographic Column - Pyrex (approximately 400 mm long X 20 mm ID) with coarse fritted plate on bottom.

4.8 Micro Syringes - 10, 25, 50 and 100 µl.

4.9 Separatory Funnel - 2000 ml, with Teflon stopcock.

4.10 Blender - High speed, glass or stainless steel cup.

4.11 Florisil - PR Grade (60-80 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

5. Reagents, Solvents and Standards

5.1 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.2 Potassium Iodide - (ACS) 10% solution in distilled water.

5.3 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.4 Sodium Sulfate - (ACS) Granular, anhydrous.

5.5 Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

5.6 Diethyl Ether - Nanograde, redistilled in glass, if necessary.

5.6.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.6.2 Decompose ether peroxides by adding 40g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.6.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.7 Hexane, Methanol, Methylene Chloride, Petroleum Ether - nanograde, redistill in glass if necessary.

5.8 Pesticide Standards - Reference grade.

5.8.1 TLC Standards - 0.100 µg/µl in chloroform.

5.9 Nitrous acid - prepare just before use by mixing 1 g $NaNO_2$ with 20 ml 0.2 N HCl.

5.10 Chromogenic agent - Dissolve 1.0 g 1-Naphthol in 20 ml ethanol. Prepare fresh daily.

6. Calibration

6.1 To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

6.2 Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

6.3 For qualitative and quantitative work, spot a series representing 0.1-1.0 µg of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

8. Sample Preparation

8.1 Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

8.2 Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

9. Extraction

9.1 Add 60 ml of methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

9.2 Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pass the organic layer through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel, and complete the extraction procedure a second time. Perform a third extraction in the same manner.

9.3 Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

9.4 If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section 11).

10. Florisil Column Cleanup

10.1 Adjust the sample extract to 10 ml with hexane.

10.2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

10.3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

15% Eluate	50% Eluate	100% Eluate
CIPC	Barban (5%)	Neburon (92%)
IPC	Linuron	Diuron
Barban (95%)	Neburon (8%)	Fluometuron
		Monuron
		Siduron
		Urox (25%)

CAUTION: Fenuron and Urab are not recovered from the Florisil column. The recovery of Urox is very poor.

10.4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 µl.

10.5 Analyze according to 11. below.

11. Separation and Detection

11.1 Carefully spot 10% of the extract on a thin layer. On the same plate spot several pesticides or mixtures for screening purposes, or a series of 1,2,4,6,8 and 10 µl of specific standards for quantitative analysis.

11.2 Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.

11.3 Spray the layer rapidly and evenly with about 10-15 ml sulfuric acid solution. Heat the layer in an oven at 110 C for 15 minutes.

11.4 When the layer is cool, spray it with nitrous acid reagent and allow it to dry. Spray the layer with 1-naphthol reagent and allow it to dry again. The pesticides will appear as purple spots (see Table 2). Identifications are made by comparison of colors and R_f values. Quantitative estimates are made by visually comparing the intensity and size of the spots with those of the series of standard.

12. Calculation of Results

12.1 Determine the concentration of pesticide in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter.

13. Reporting Results

13.1 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed

REFERENCES

(1) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(2) "Methods for Organic Pesticides in Water and Wastewater," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.

(3) Katz, S. E., "Determination of the Substituted Urea Herbicides Linuron, Monuron, Diuron, Neburon, and Fenuron in Surface Water," Journal of the Association of Official Agricultural Chemists, 49, 452 (1966).

(4) Geissbuhler, H. and Gross, D., "Specific Detection of Urea Herbicide Residues by Separation of Their Amines on Cellulose Thin-Layer Plates," Journal of Chromatography, 27, 296 (1967).

(5) Askew, J. et al., "Use of Hydriodic Acid in the Detection of Pesticides After Thin-Layer Chromatography," Journal of Chromatography, 37, 369 (1968).

(6) Stahl, E., "Thin-Layer Chromatography," Academic Press, New York, 1969.

(7) Longbottom, J. E. and Lichtenberg, J. J., "Determination of Carbamate and Urea Pesticides in Surface Waters by Thin-Layer Chromatography," U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

(8) Steere, N. V., editor, "Handbook for Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

TABLE 1

R_f VALUES OF N-ARYL CARBAMATE AND UREA PESTICIDES IN SEVERAL SOLVENT SYSTEMS

Carbamates	A	B	C	D	E	F	G
Propham	0.49	0.54	0.73	0.48	0.36	0.68	0.69
Chlorpropham	0.57	0.60	0.73	0.49	0.37	0.70	0.73
Barban	0.61	0.59	0.72	0.41	0.28	0.70	0.74
Swep	0.48	0.44	0.70	0.41	0.28	0.67	0.66
Urea							
Fenuron	0.03	0.04	0.38	0.22	0.10	0.41	0.30
Urab	0.03	0.04	0.36	0.22	0.10	0.41	0.30
Monuron	0.04	0.05	0.37	0.24	0.10	0.47	0.34
Urox	0.04	0.06	0.34	0.24	0.10	0.46	0.34
Diuron	0.05	0.09	0.38	0.28	0.13	0.54	0.44
Linuron	0.40	0.43	0.62	0.39	0.24	0.66	0.64
Neburon	0.21	0.28	0.64	0.41	0.26	0.68	0.65
Siduron	0.02	0.07	0.68	0.39	0.25	0.62	0.55

Solvent Systems

A. Methylene chloride
B. Chloroform
C. Ethyl Acetate
D. Hexane/acetone (2:1)
E. Hexane/acetone (4:1)
F. Chloroform/acetonitrile (2:1)
G. Chloroform/acetonitrile (5:1)

TABLE 2

COLOR RESPONSES AND DETECTION LIMIT FOR THE

N-ARYL CARBAMATES AND UREAS

Carbamates	Color	Detection Limit (µg)
Propham	Red-purple	0.2
Chlorpropham	Purple	0.1
Barban	Purple	0.05
Swep	Blue-Purple	0.2
Ureas		
Fenuron	Red-purple	0.05
Urab	Red-purple	0.1
Monuron	Pink-orange	0.05
Urox	Pink-orange	0.1
Diuron	Blue-purple	0.1
Linuron	Blue-purple	0.1
Neburon	Blue-purple	0.1
Siduron	Red-purple	0.05

APPENDIX I

3. Standardization of Florisil Column by Weight Adjustment Based on Adsorption of Lauric Acid.

13.1 A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

13.2 Apparatus

13.2.1 Buret. -- 25 ml with 1/10 ml graduations.

13.2.2 Erlenmeyer flasks. -- 125 ml narrow mouth and 25 ml, glass stoppered.

13.2.3 Pipet. -- 10 and 20 ml transfer.

13.2.4 Volumetric flasks. -- 500 ml.

13.3 Reagents and Solvents

13.3.1 Alcohol, ethyl. -- USP or absolute, neutralized to phenolphthalein.

13.3.2 Hexane. -- Distilled from all glass apparatus.

13.3.3 Lauric acid. --Purified, CP.

13.3.4 Lauric acid solution. -- Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).

13.3.5 Phenolphthalein Indicator. -- Dissolve 1 g in alcohol and dilute to 100 ml.

13.3.6 Sodium hydroxide. -- Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

13.4 Procedure

13.4.1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

13.4.2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

13.5 Calculation of Lauric Acid Value and Adjustment of Column Weight

13.5.1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).

13.5.2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

13.6 Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

☆ U.S. GOVERNMENT PRINTING OFFICE 1973— 759-555/1150

7. METHOD FOR CHLORINATED PHENOXY ACID HERBICIDES IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method covers the determination of chlorinated phenoxy acid herbicides in industrial effluents. The compounds 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), 2,3-dichloro-o-anisic acid (dicamba) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may be determined by this procedure.

1.2 Since these compounds may occur in water in various forms (i.e., acid, salt, ester, etc.) a hydrolysis step is included to permit the determination of the active part of the herbicide. The method may be applied to additional phenoxy acids and certain phenols. However, the analyst must demonstrate the usefulness of the method for each specific compound before applying it to sample analysis.

2. Summary

2.1 Chlorinated phenoxy acids and their esters are extracted from the acidified water sample with ethyl ether. The esters are hydrolyzed to acids and extraneous organic material is removed by a solvent wash. The acids are converted to methyl esters which are extracted from the aqueous phase. The extract is cleaned up by passing it through a micro-adsorption column. Identification of the esters is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography (1). Results are reported in micrograms per liter.

2.2 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part 1, Sections 1.4 and 1.5, (2).

3.2 The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of chlorinated phenoxy acid herbicides. Sample clean-up procedures are generally required and may result in loss of certain of these herbicides. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

3.3 Organic acids, especially chlorinated acids, cause the most direct interference with the determination. Phenols including chlorophenols will also interfere with this procedure.

3.4 Alkaline hydrolysis and subsequent extraction eliminates many of the predominant chlorinated insecticides which might otherwise interfere with the test.

3.5 The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Glassware and glass wool should be acid-rinsed and sodium sulfate should be acidified with sulfuric acid to avoid this possibility.

4. Apparatus and Materials

4.1 Gas Chromatograph - Equipped with glass lined injection port.

4.2 Detector Options:

4.2.1 Electron Capture - Radioactive (tritium or nickel-63)

4.2.2 Microcoulometric Titration

4.2.3 Electrolytic Conductivity

4.3 Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

4.4 Gas Chromatographic Column Materials:

4.4.1 Tubing - Pyrex (180 cm long X 4 mm ID)

4.4.2 Glass Wool - Silanized

4.4.3 Solid Support - Gas-Chrom-Q (100-120 mesh)

4.4.4 Liquid Phases - Expressed as weight percent coated on solid support.

4.4.4.1 OV-210, 5%

4.4.4.2 OV-17, 1.5% plus QF-1, 1.95%

4.5 Kuderna-Danish (K-D) Glassware (Kontes)

4.5.1 Snyder Column - three ball (macro) and two ball (micro)

4.5.2 Evaporative Flasks - 250 ml

4.5.3 Receiver Ampuls - 10 ml, graduated

4.5.4 Ampul Stoppers

4.6 Blender High speed, glass or stainless steel cup.

4.7 Graduated cylinders - 100 and 250 ml.

4.8 Erlenmeyer flasks 125 ml, 250 ml ground glass $\overline{S}$ 24/40

4.9 Microsyringes - 10, 25, 50 and 100 µl.

4.10 Pipets - Pasteur, glass disposable (140 mm long X 5 mm ID).

4.11 Separatory Funnels - 60 ml and 2000 ml with Teflon stopcock.

4.12 Glass wool - Filtering grade, acid washed.

4.13 Diazald Kit recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. #210,025-2)

4.14 Florisil - PR grade (60-100 mesh) purchased activated at 1250F and stored at 130 C.

5. Reagents, Solvents and Standards

5.1 Boron Trifluoride-Methanol-esterification-reagent, 14 percent boron trifluoride by weight.

5.2 N-methyl-N-nitroso-p-toluenesulfonamide (Diazald) - High purity, melting point range 60-62 C. Precursor for the generation of diazomethane (see Appendix I).

5.3 Ferrous Sulfate - (ACS) 30% solution in distilled water.

5.4 Potassium Hydroxide Solution - A 37 percent aqueous solution prepared from reagent grade potassium hydroxide pellets and reagent water.

5.5 Potassium Iodide - (ACS) 10% solution in distilled water.

5.6 Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

5.7 Sodium Hydroxide - (ACS) 10 N in distilled water.

5.8 Sodium Sulfate, Acidified. -- (ACS) granular sodium sulfate, treated as follows: Add 0.1 ml of conc. sulfuric acid to 100 g of sodium sulfate slurried with enough ethyl ether to just cover the solid. Remove the ether with the vacuum. Mix 1 g of the resulting solid with 5 ml of reagent water and ensure the mixture to have a pH below 4. Store at 130 C.

5.9 Sulfuric acid. -- (ACS) concentrated, Sp. Gr. 1.84.

5.9.a. Carbitol (diethylene glycol monoethyl ether).

5.10 Diethyl Ether Nanograde, redistilled in glass, if necessary.

5.10.1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

5.10.2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

5.10.3 Distill deperoxidized ether in glass and add 2% ethanol.

5.11 Benzene Hexane Nanograde, redistilled in glass, if necessary.

5.12 Pesticide Standards - Acids and Methyl Esters, reference grade.

5.12.1 Stock standard solutions - Dissolve 100 mg of each herbicide in 60 ml ethyl ether; then make to 100 ml with redistilled hexane. Solution contains 1 mg/ml.

5.12.2 Working standard - Pipet 1.0 ml of each stock soln into a single 100 ml volumetric flask. Make to volume with a mixture of ethyl ether and hexane (1:1). Solution contains 10 µg/ml of each standard.

5.12.3 Standard for Chromatography - (Diazomethane Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube and evaporate off the solvent using steam bath. Add 2 ml diazomethane to the residue. Let stand 10 minutes with occasional shaking, then allow the solvent to evaporate spontaneously. Dissolve the residue in 200 µl of hexane for gas chromatography.

5.12.4 Standard for Chromatography -(Boron Trifluoride Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube. Add 0.5 ml of Benzene and evaporate to 0.4 ml using a two-ball Snyder microcolumn and a steam bath. Proceed as in 11.3.1. Esters are then ready for gas chromatography.

6. Calibration

6.1 Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when $\leq$ 0.06 ng is injected for electron capture detection and $\leq$ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

6.2 Standards, prepared from methyl esters of phenoxy acid herbicides calculated as the acid equivalent, are injected frequently as a check on the stability of operating conditions.

6.3 The elution order and retention ratios of methyl esters of chlorinated phenoxy acid herbicides are provided in Table 1, as a guide.

7. Quality Control

7.1 Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed the use of quality control charts is recommended (3).

7.2 Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

8. Sample Preparation

8.1 Blend the sample, if suspended matter is present.

8.2 For a sensitivity requirement of 1 µg/l, when using electron capture for detection, take 100 ml of sample for analysis. For microcoulometric or electrolytic conductivity detection, take 1-liter of sample. Background information on the extent and nature of interferences will assist the analyst in selecting the proper sample size and detector.

8.3 Quantitatively transfer the proper aliquot of sample into a two-liter separatory funnel, dilute to one liter and acidify to approximately pH 2 with concentrated sulfuric acid. Check pH with indicator paper.

9. Extraction

9.1 Add 150 ml of ether to the sample in the separatory funnel and shake vigorously for one minute.

9.2 Allow the contents to separate for at least ten minutes. After the layers have separated, drain the water phase into a one-liter Erlenmeyer flask. Then collect the extract in a 250 ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.

9.3 Extract the sample two more times using 50 ml of ether each time, and combine the extracts in the Erlenmeyer flask. (Rinse the one-liter flask with each additional aliquot of extracting solvent.)

10. Hydrolysis

10.1 Add 15 ml of distilled water and a small boiling stone to the flask containing the ether extract, and fit the flask with a 3-ball Snyder column. Evaporate the ether on a steam bath and continue heating for a total of 60 minutes.

10.2 Transfer the concentrate to a 60 ml separatory funnel. Extract the basic solution two times with 20 ml of ether and discard the ether layers. The herbicides remain in the aqueous phase.

10.3 Acidify the contents of the separatory funnel by adding 2 ml of cold (4 C) 25 percent sulfuric acid (5.9). Extract the herbicides once with 20 ml of ether and twice with 10 ml of ether. Collect the extracts in a 125 ml Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate (5.8). Allow the extract to remain in contact with the sodium sulfate for approximately two hours.

11. Esterification (4,5)

11.1 Transfer the ether extract, through a funnel plugged with glass wool, into a Kuderna-Danish flask equipped with a 10 ml graduated ampul. Use liberal washings of ether. Using a glass rod, crush any caked sodium sulfate during the transfer.

11.1.1 If esterification is to be done with diazomethane, evaporate to approximately 4 ml on a steam bath (do not immerse the ampul in water) and proceed as directed in Section 11.2.

11.1.2 If esterification is to be done with boron trifluoride, add 0.5 ml benzene and evaporate to about 5 ml on a steam bath. Remove the ampul from the flask and further concentrate the extract to 0.4 ml using a two-ball Snyder microcolumn and proceed as in 11.3.

11.2 Diazomethane Esterification

11.2.1 Disconnect the ampul from the K-D flask and place in a hood away from steam bath. Adjust volume to 4 ml with ether, add 2 ml diazomethane, and let stand 10 minutes with occasional swirling.

11.2.2 Rinse inside wall of ampul with several hundred microliters of ethyl ether. Take sample to approximately 2 ml to remove excess diazomethane by allowing solvent to evaporate spontaneously (room temperature).

11.2.3 Dissolve residue in 5 ml of hexane. Analyze by gas chromatography.

11.2.4 If further clean-up of the sample is required, proceed as in 11.3.4 substituting hexane for benzene.

11.3 Boron Trifluoride Esterification

11.3.1 After the benzene solution in the ampul has cooled, add 0.5 ml of borontrifluoride-methanol reagent. Use the two-ball Snyder micro column as an air-cooled condenser and hold the contents of the ampul at 50 C for 30 minutes on the steam bath.

11.3.2 Cool and add about 4.5 ml of a neutral 5 percent aqueous sodium sulfate solution so that the benzene-water interface is in the neck of the Kuderna-Danish ampul. Seal the flask with a ground glass stopper and shake vigorously for about one minute. Allow to stand for three minutes for phase separation

11.3.4 Pipet the solvent layer from the ampul to the top of a small column prepared by plugging a disposable Pasteur pipet with glass wool and packing with 2.0 cm of sodium sulfate over 1.5 cm of Florisil adsorbent. Collect the eluate in a graduated ampul. Complete the transfer by repeatedly rinsing the ampul with small quantities of benzene and passing the rinses through the column until a final volume of 5.0 ml of eluate is obtained. Analyze by gas chromatography.

12. Calculation of Results

12.1 Determine the methyl ester concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2 (2).

(1) $$\text{Micrograms/liter} = \frac{(A)\quad(B)\quad(V_t)}{(V_i)\quad(V_s)}$$

$A = \frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_l = Volume of extract injected (µl)

V_t = Volume of total extract (µl)

V_s = Volume of water extracted (ml)

12.2 Molecular weights for the calculation of the methyl esters as the acid equivalents.

2,4-D	222.0	Dicamba	221.0
2,4-D Methyl ester	236.0	Dicamba methyl ester	236.1
Silvex	269.5	2,4,5-T	255.5
Silvex methyl ester	283.5	2,4,5-T methyl ester	269.5

13. Reporting Results

13.1 Report results in micrograms per liter as the acid equivalent without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

Table 1

RETENTION RATIOS FOR METHYL ESTERS OF SOME CHLORINATED PHENOXY ACID HERBICIDES RELATIVE TO 2,4-D

Liquid Phase[1]	1.5% OV-17 + 2.95% QF-1	5% OV-210
Column Temp.	185 C	185 C
Argon/Methane Carrier Flow	70 ml/min	70 ml/min
Herbicide	RR	RR
2,4-D	1.00	1.00
silvex	1.34	1.22
2,4,5-T	1.72	1.51
dicamba	0.60	0.61
2,4-D (minutes absolute)	2.00	1.62

[1]All columns glass, 180 cm X 4 mm ID, solid support Gas Chrom Q (100/120 mesh)

REFERENCES

(1) Goerlitz, D. G., and Lamar, W. L., "Determination of Phenoxy and Herbicides in Water by Electron-Capture and Microcoulometric Gas Chromatography", U. S. Geol. Survey Water-Supply Paper 1817-C (1967).

(2) "Methods for Organic Pesticides in Water and Wastewater", (1971) U. S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio.

(3) "Handbook for Analytical Quality Control in Water and Wastewater Laboratories" (1972), U. S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268.

(4) Metcalf, L. D., and Schmitz, A. A., "The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis", Analytical Chemistry, 33, 363 (1961).

(5) Schlenk, H. and Gellerman, J. L., "Esterification of Fatty Acids with Diazomethane on a Small Scale", Analytical Chemistry, 32, 1412 (1960).

(6) "Pesticide Analytical Manual", U. S. Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.

(7) Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

APPENDIX I

Diazomethane in ether (6).

1. CAUTIONS. Diazomethane is very toxic. It can explode under certain conditions. The following precautions should be observed.

Avoid breathing vapors.

Use only in well-ventilated hood.

Use safety screen.

Do not pipette solution of diazomethane by mouth.

For pouring solutions of diazomethane, use of gloves is optional.

Do not heat solutions to 100 C (EXPLOSIONS).

Store solutions of gas at low temperatures (Freezer compartment of explosion proof refrigerators).

Avoid ground glass apparatus, glass stirrers and sleeve bearings where grinding may occur (EXPLOSIONS).

Keep solutions away from alkali metals (EXPLOSIONS).

Solutions of diazomethane decompose rapidly in presence of solid material such as copper powder, calcium chloride, boiling stones, etc. These solid materials cause solid polymethylene and nitrogen gas to form.

2. PREPARATION.

Use a well-ventilated hood and cork stoppers for all connections. Fit a 125 ml long-neck distilling flask with a dropping funnel and an efficient condenser set downward for distillation. Connect the condenser to two receiving flasks in series a 500 ml Erlenmeyer followed by a 125 ml Erlenmeyer containing 30 ml ether. The inlet to the 125 ml Erlenmeyer should dip below the ether. Cool both receivers to 0 C.

As water bath for the distilling flask, set up a 2-liter beaker on a stirplate (hot plate and stirrer), maintaining temperature at 70 C.

Dissolve 6 g KOH in 10 ml water in the distilling flask (no heat). Add 35 ml Carbitol (diethylene glycol monoethyl ether), stirring bar, and another 10 ml ether. Connect the distilling flask to the condenser and immerse distilling flask in water bath. By means of the dropping funnel, add a solution of 21.5 g Diazald in 140 ml ether over a period of 20 minutes. After distillation is apparently complete, add another 20 ml ether and continue distilling until distillate is colorless. Combine the contents of the two receivers in a glass bottle (WITHOUT ground glass neck), stopper with cork, and freeze overnight. Decant the diazomethane from the ice crystals into a glass bottle, stopper with cork, and store in freezer until ready for use. The final solution may be stored up to six months without marked deterioration.

The 21.5 g of Diazald reacted in this manner produce about 3 g of Diazomethane.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1151

Fed. Reg., 38, No. 75, Pt. II

8. A METHOD FOR ORGANOCHLORINE SOLVENTS IN INDUSTRIAL EFFLUENTS

1. Scope and Application

1.1 This method describes a direct aqueous-injection (1)(2) procedure for the determination of gas chromatographable chlorinated hydrocarbons. The method is specific for hydrocarbons containing chlorine, iodine, and bromine. It is sensitive to approximately 1 mg/l. The compounds detected are composed of carbon, the above mentioned halogens and usually hydrogen; however, these compounds may also contain fluorine, oxygen, nitrogen, sulfur or phosphorus.

1.2 The method is useful only for organo-halide compounds with a water solubility exceeding 1 mg/l @ 22°C. Many commonly used organochlorine solvents meet this specification, i.e., carbon tetrachloride, 800 mg/l at 20°C, chloroform, 1000 mg/l at 15°C, methylene chloride, 2000 mg/l at 20°C (3).

2. Summary

2.1 If the sample is turbid it is initially centrifuged or filtered through a fiber glass filter in order to remove suspended matter. A three to ten microliter aliquot of the sample is injected into the gas chromatograph equipped with a halogen specific detector. The resulting chromatogram is used to identify and quantitate specific components in the sample. Results are reported in micrograms per liter. Confirmation of qualitative identifications are made using two or more dissimilar columns.

3. Sample Collection and Handling

3.1 A representative sample should be collected in a clean glass bottle. Containers such as a BOD bottle or a 1-quart wide mouth bottle with a TFE fluorocarbon cap liner should be used.

3.2 The sample volume should be in excess of 200 ml.

3.3 The sample is best preserved by protecting it from phase separation. Since the majority of the chlorinated solvents are volatile and relatively insoluble in water, it is important that the sample bottle be filled completely to minimize air space over the sample. The sample must remain hermetically sealed up to the time it is analyzed. Refrigeration or freezing only encourages phase separation and should be avoided. Acidification will minimize the formation of non-volatile salts formed from chloroorganic acids and certain chlorophenols. However, it may interfere with the detection of acid degradable compounds such as chloroesters. Therefore, the sample history must be known before any chemical or physical preservation steps can be applied. To insure sample integrity, it is best to analyze the sample within 1 hour of collection.

4. Interferences

4.1 The use of a halogen specific detector eliminates any possibility of interference from compounds not containing chlorine, bromine, or iodine. Compounds containing bromine or iodine will interfere with the determination of organochlorine compounds. The use of two dissimilar chromatographic columns helps to minimize this interference and in addition this procedure helps to verify all qualitative identifications. When concentrations are sufficiently high, unequivocal identifications can be made using infrared or mass spectroscopy. Though non-specific, the flame ionization detector may be used for known systems where interferences are not a problem.

4.2 Ghosting is usually attributed to the history of the chromatographic system. Each time a sample is injected small amounts of various compounds are adsorbed on active sites in the inlet and at the head of

the column. Subsequent injections of water tend to steam clean these sites resulting in non-representative peaks or displacement of the baseline. This phenomenom normally occurs when an analysis of a series of highly concentrated samples is followed by a low level analysis. The system should be checked for ghost peaks prior to each quantitative analysis by injecting distilled water in a manner identical to the sample analysis (5). If excessive ghosting occurs, the following maintenance should be applied, as required, in the order listed:

1) Multiple flushes with distilled water
2) Clean or replace the glass injector liner
3) Replace the chromatographic column

5. Apparatus and Materials

5.1 Gas Chromatograph Equipped with programmed oven temperature controls and glass-lined injection port. The oven should be equipped with a column exit port and heated transfer line for convenient attachment to the halogen specific detector.

5.2 Detector Options:

5.2.1 Microcoulometric Titration

5.2.2 Electrolytic Conductivity

5.2.3 Flame Ionization

5.3 Recorder - Potentiometric strip chart recorder (10 in) compatible with the detector.

5.4 Syringes - 1 µl, 10 µl, and 50 µl.

5.5 BOD type bottle or 1 quart bottle with Teflon lined screw cap.

5.6 Volumetric Flasks - 500 ml, 1000 ml.

5.7 Syringe Hypodermic Lur-lock type (30 ml).

5.8 Filter glass fiber filter - Type A (13 mm).

5.9 Filter holder - Swinny type hypodermic adapter (13 mm).

5.10 Glass stoppered ampuls - 10 ml

5.10.1 Non-Polar Column - 12 ft x 0.1 in ID x 0.125 in OD stainless steel column #304 packed with 5% OV-1 on chromosorb-W (60-80 mesh).

5.10.2 Moderately-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel column #304 packed with 5% carbowax 20 M on Chromosorb-W (60-80 mesh).

5.10.3 Highly-Polar Column - 23 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with 5% 1,2,3-Tris-(2-cyanoethoxy) propane on Chromosorb-W (60-80 mesh).

5.10.4 Porous Polymer Column - 6 ft x 0.1 in ID x 0.125 in OD stainless steel #304 packed with Chromosorb-101 (60-80 mesh).

6. Reagents

6.1 Chlorinated hydrocarbons reference standards

6.1.1 Prepare standard mixtures in volumetric flasks using contaminant free distilled water as solvent. Add a known amount of the chlorinated compounds with a microliter syringe. Calculate the concentration of each component as follows:

$$\text{mg/l} = (\text{Density of Compound})(\mu\text{l injected})\left\{\frac{1000}{(\text{Dilution Volume (ml)})}\right\}$$

7. Quality Control

7.1 Duplicate quantitative analysis on dissimilar columns should be performed. The duplicate quantitative data should agree within experimental error (±6 percent). If not, analysis on a third dissimilar column should be performed. Spiked sample analyses should be routinely performed to insure the integrity of the method.

8. Selection Gas Chromatographic Column

8.1 No single column can efficiently resolve all chlorinated hydrocarbons. Therefore, a specific column must be selected to perform a given analysis. Columns providing only partially or non-resolved peaks are useful only for confirmatory identifications. If the qualitative nature of the sample is known then an efficient column selection can be made by reviewing literature (4). In doing this, one must remember that injection of large volumes of water can cause two serious problems not normally noted using common gas chromatographic techniques:

1) Water can cause early column failure due to liquid phase displacement.

2) Water passing through the column causes retention times and orders to change when compared to common sample solvent media, ie., hexane or air.

For these reasons column life and the separations obtained by direct aqueous injection may not be identical to those suggested in literature.

8.2 If nothing is known about the sample, a thermally stable non-polar column such as the OV-1 column is a good first choice. Temperature programming this column from room temperature to its upper limit will provide a wide molecular weight range analysis. Following this with a moderately polar column and a highly polar column provides efficient separations and corroborative identifications for a wide variety of common chlorinated solvents. The unique low molecular weight separations achieved on porous polymer columns is extremely useful when the samples contain a mixture of such compounds.

9. Sample Preparation

9.1 If the sample is turbid it should be filtered or contrifuged to prevent syringe plugging or excessive ghosting problems. Filtering the sample is accomplished by filling a 30 ml hypodermic syringe with sample and attaching the Swinny type hypodermic filter adaptor with a glass fiber filter "Type A" installed. Discard the first 5 ml of sample then collect the filtered sample in a glass stoppered ampule filled to the top. (One should occasionally analyze the non-filtered sample to insure that the filtering technique does not adversely effect the sample).

10. Method of Analysis

10.1 First, analyze the filtered sample of unknown composition by injection of a 3 to 10 µl into the gas chromatograph. The injection volume and detector sensitivity is recorded.

10.2 Prepare a standard mixture consisting of the same compounds in concentrations approximately equal to those detected in the sample. Chromatograph the standard mixture under conditions identical to the unknown.

11. Calculation of Results

11.1 Measure the area of each unknown peak and each reference standard peak as follows:

Area = [Peak Height][Width of Peak at 1/2 Height]

11.2 Calculate the concentration of each unknown as follows:

$$\text{mg/l} = \frac{(\text{Area of Sample peak})(\mu\text{l of Standard Injected})(\text{Conc'n of Standard})}{(\mu\text{l of Sample Injected})(\text{Area of Standard Peak})}$$

12. Reporting Results

12.1 Report results in mg/l. If a result is negative, report the minimum detectable limit, ie. <1 mg/l. When duplicate and spiked samples are analyzed, all data obtained should be reported.

References

1. "Tentative Recommended Practice for Measuring Volatile Organic Matter in Water by Aqueous - Injection Gas Chromatography", D2908-70T, 1971 Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis, American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

2. Bellar, T. A. and Lichtenberg, J. J., "Method for the Determination of Chlorinated Organic Solvents by Direct Aqueous Injection Gas Chromatography", U. S. Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268 (March 1973).

3. "Handbook of Chemistry and Physics", 48th Edition, The Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128. (1967-1968)

4. "Gas Chromatography Abstracts", Knapman, C.E.H., Editor, Institute of Petroleum, 61 New Cavendish Street, London W1M8AR, Annually 1958 to date, since 1970, also includes Liquid Chromatography Abstracts.

5. Dressman, R. C., "Elimination of Memory Peaks Encountered in Aqueous-Injection Gas Chromatography", Journal of Chromatographic Science, 8, 265 (1970).

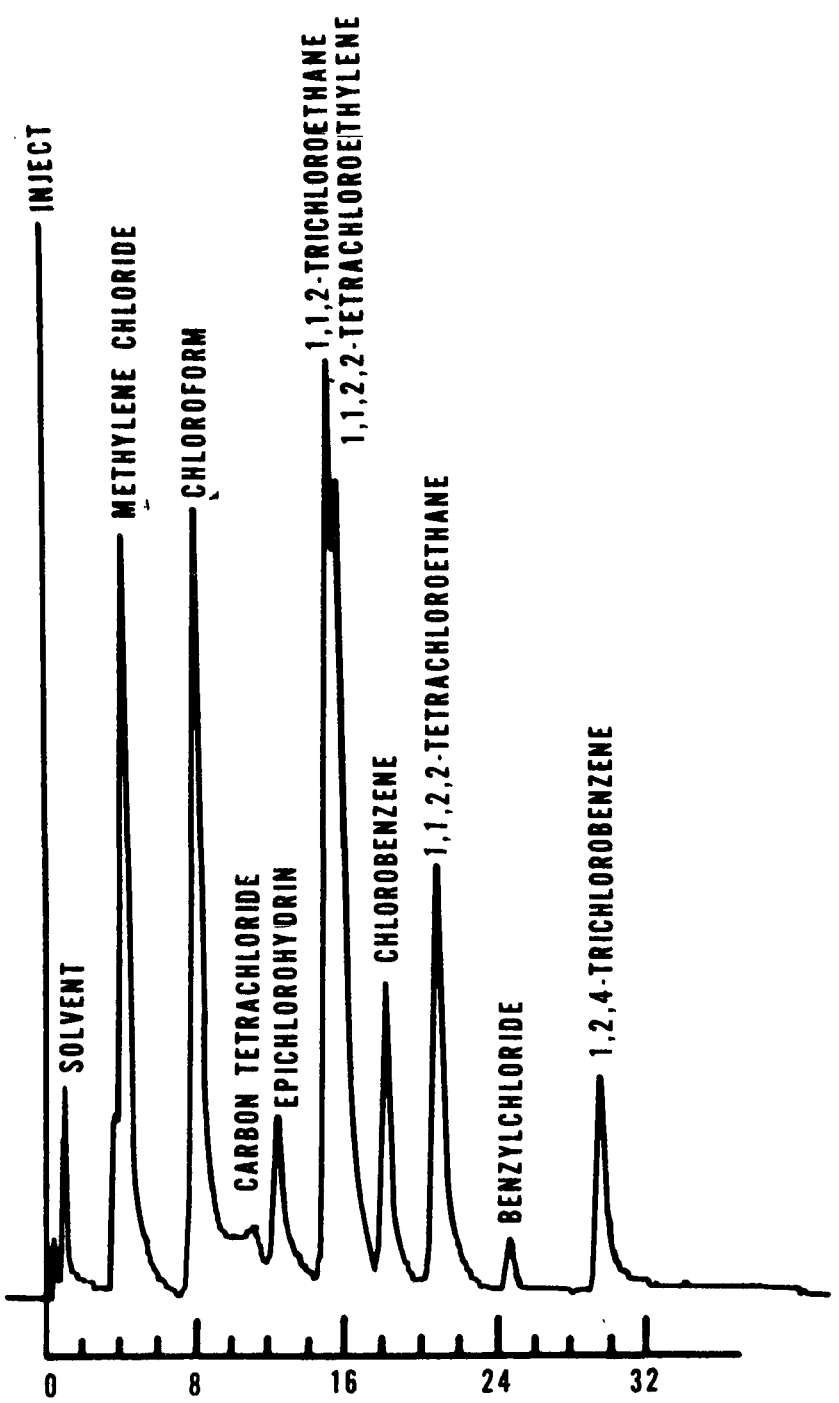


Figure 1. Column: Chromosorb-101, Temperature Program: 125 C for 4 min then 4 C/min up to 280 C., Carrier Gas: Nitrogen at 36 ml/min, Detector: Microcoulometric.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1152

ANTIMONY

STORET NO:
(Standard Conditions) TOTAL : 01097

Optimum Concentration Range: 1-40 mg/l using a wavelength of 217.6 nm

Sensitivity: 0.3 mg/l

Detection Limit: 0.2 mg/l

Preparation of Standard Solution:

1. Stock Solution: Carefully weigh 2.7426 grams of antimony potassium tartrate (analytical reagent grade) and dissolve in distilled water. Dilute to 1 liter with distilled water. One ml equals 1 mg Sb (1000 mg/l).
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis.

Sample Preparation:

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes, 1971" (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General):

1. Antimony hollow cathode lamp.
2. Wavelength: 217.6 nm
3. Type of burner: Boling.
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of flame: Fuel rich
7. Photomultiplier tube: R106

Interferences:

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. In the presence of lead (1000 mg/l), a spectral interference may occur at the 217.6 nm resonance line. In this case the 231.1 nm antimony line should be used.
3. Increasing acid concentrations decrease antimony absorption. To avoid this effect, the acid concentrations in the samples and in the standards should be matched.

Notes:

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

BARIUM

STORET NO:

(Standard Conditions) TOTAL : 01007

Optimum Concentration Range 0.5-20 mg/l using a wavelength of 553.6 nm

Sensitivity 0.2 mg/l

Detection Limit 0.03 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 1.7787 g barium chloride ($BaCl_2 \cdot 2H_2O$, analytical reagent grade) in distilled water and dilute to 1 liter. One ml equals 1 mg Ba.
2. Potassium chloride solution: Dissolve 95g potassium chloride, KCl, in distilled water and make up to 1 liter.
3. Prepare dilutions of the stock barium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike add 2.0 ml potassium chloride solution.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General)

1. Barium hollow cathode lamp
2. Wavelength: 553.6 nm
3. Type of burner: Nitrous oxide
4. Fuel: Acetylene
5. Oxidant: Nitrous oxide
6. Type of flame: Fuel rich
7. Photomultiplier tube: 1P28

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. The use of a nitrous oxide-acetylene flame virtually eliminates chemical interference; however, barium is easily ionized in this flame and potassium must be added (1000 mg/l) to standards and samples alike to control this effect.
3. If the nitrous oxide flame is not available and acetylene-air is used, phosphate, silicon, and aluminum will severely depress the barium absorbance. This may be overcome by the additon of 2000 mg/l lanthanum.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

BERYLLIUM

STORET NO:

(Standard Conditions) TOTAL : 01012

Optimum Concentration Range: 0.02-1.5 mg/l using a wavelength of 234.9 nm

Sensitivity: 0.007 mg/l.

Detection Limit: 0.002 mg/l.

Preparation of Standard Solution:

1. Stock solution: Dissolve 11.6586 g beryllium sulfate, $BeSO_4$, in distilled water containing 2 ml conc. nitric acid and dilute to 1 liter. One ml equals 1 mg Be.
2. Prepare dilutions of the stock solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% nitric acid in all calibration standards.

Sample Preparation:

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes, 1971" (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General):

1. Beryllium hollow cathode lamp
2. Wavelength: 234.9 nm
3. Type of burner: Nitrous oxide
4. Fuel: Acetylene
5. Oxidant: Nitrous oxide
6. Type of flame: Fuel rich
7. Photomultiplier tube R 106

Interferences:

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Sodium and silicon at concentrations in excess of 1000 mg/l have been found to severely depress the beryllium absorbance.
3. Bicarbonate ion is reported to interfere, however, its effect is eliminated when samples are acidified to a pH of 1.5.

Notes:

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

BORON

(Curcumin Method)

STORET NO:
TOTAL : 01022

1. Scope and Application

1.1 This colorimetric method finds maximum utility for waters whose boron content is below 1 mg/l.

1.2 The optimum range of the method on undiluted or unconcentrated samples is 0.1-1.0 mg/l of boron.

2. Summary of Method

1.1 When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent, and the red color is compared with standards either visually or photometrically.

3. Comments

3.1 Nitrate nitrogen concentrations above 20 mg/l interfere.

3.2 Significantly high results are possible when the total of calcium and magnesium hardness exceeds 100 mg/l as $CaCO_3$. Passing the sample through a cation exchange resin eliminates this problem.

3.3 Close control of such variables as volumes and concentrations of reagents, as well as time and temperature of drying, must be exercised for maximum accuracy.

4. Precision and Accuracy

4/1 A synthetic unknown sample containing 240 µg/l B, 40 µg/l As, 250 µg/l Be, 20 µg/l Se, and 6 µg/l V in distilled water was

determined by the curcumin method with a relative standard deviation of 22.8% and a relative error of 0% in 30 laboratories.

5. Reference

5.1 The procedure to be used for this determination is found in: Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 69, Method 107A (1971).

6. Data to be entered into STORET must be reported as µg/l.

COBALT

STORET NO:
(Standard Conditions) TOTAL : 01037

Optimum Concentration Range: 0.2-7 mg/l using a wavelength of 240.7 nm.

Sensitivity: 0.05 mg/l

Detection Limit: 0.02 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 4.037 grams of cobaltous chloride, $CoCl_2 \cdot 6H_2O$ (analytical reagent grade) in distilled water. Add 10 ml of concentrated nitric acid and dilute to 1 liter with distilled water. One ml equals 1 mg Co (1000 mg/l).
2. Prepare dilutions of the stock cobalt solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% nitric acid in all calibration standards.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General)

1. Cobalt hollow cathode lamp.
2. Wavelength: 240.7 nm
3. Type of burner: Boling
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of Flame: Stoichiometric
7. Photomultiplier tube: R-106

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Interference from high concentrations (1000 mg/l) of calcium, aluminum, potassium, magnesium, phosphate, sulfate, nitrate and silicate amy be observed. The use of the nitrous oxide-acetylene flame will lessen this interference with some loss of sensitivity.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. With the exception of certain samples and/or effluents containing high levels of extractable metals, the APDC-MIBK extraction technique should be used for concentrations below 20 µg/l.
3. Data to be entered into STORET must be reported as µg/l.

MOLYBDENUM

STORET NO:

(Standard Conditions) TOTAL : 01062

Optimum Concentration Range 0.4-20 mg/l using a wavelength of 313.3 nm.

Sensitivity 0.1 mg/l

Detection Limit 0.03 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 1.840 grams of ammonium molybdate $(NH_4)_6\ Mo_7O_{24}\cdot 4H_2O$ (analytical reagent grade) in distilled water and dilute to 1 liter. One ml equals 1 mg Mo (1000 mg/l).
2. Prepare dilutions of the stock molybdenum solution to be used as calibration standards at the time of analysis.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General)

1. Molybdenum hollow cathode lamp
2. Wavelength: 313.3 nm
3. Type of burner: Nitrous oxide
4. Fuel: Acetylene
5. Oxidant: Nitrous Oxide
6. Type of flame: Fuel rich
7. Photomultiplier tube: 1P28

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a

non-absorbing wavelength should be checked.

2. With the recommended nitrous oxide-acetylene flame, interferences may be suppressed by adding 1000 mg/l of a refractory metal such as aluminum. This should be done to both samples and standards alike.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. For low levels of molybdenum an oxine extraction procedure may be useful. (Ref: Chau et.al., Anal. Chem. Acta 48 205, 1969).
3. Data to be entered into STORET must be reported as µg/l.

NICKEL

STORET NO:
(Standard Conditions) TOTAL : 01067

Optimum Concentration Range 0.2-7 mg/l using a wavelength of 232.0 nm

Sensitivity 0.05 mg/l

Detection Limit 0.01 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 4.953 grams of nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$ (analytical reagent grade) in distilled water. Add 10 ml of concentrated nitric acid and dilute to 1 liter with distilled water. One ml equals 1 mg Ni (1000 mg/1).
2. Prepare dilutions of the stock nickel solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% nitric acid in all calibration standards.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General)

1. Nickel hollow cathode lamp.
2. Wavelength: 232.0 nm
3. Type of burner: Boling
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of Flame: Oxidizing
7. Photomultiplier Tube: R 106

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering if background correction is not available, a non-absorbing wavelength should be checked.
2. The 352.4 nm wavelength is less susceptible to non-atomic absorbance and may be used. The calibration curve is more linear at this wavelength; however, there is some loss of sensitivity.
3. Interference from high concentrations (1000 mg/l) of calcium, aluminum, potassium, magnesium, phosphate, sulfate, nitrate and silicate may be observed. The use of the nitrous oxide-acetylene flame will lessen this interference with some loss of sensitivity.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. With the exception of certain samples and/or effluents containing high levels of extractable metals, the APDC-MIBK extraction technique should be used for concentrations below 20 µg/l
3. Data to be entered into STORET must be reported as µg/l.

SILVER

STORET NO:

(Standard Conditions) TOTAL : 01077

Optimum Concentration Range 0.1-20 mg/l using a wavelength of 328.1 nm.

Sensitivity 0.05 mg/l

Detection Limit 0.01 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 1.575 g of $AgNO_3$ (analytical reagent grade) in distilled water, add 10 ml HNO_3 and make up to 1 liter. One ml equals 1 mg of silver (1000 mg/l).
2. Prepare diltuions of the stock solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% HNO_3 in all calibration standards.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) has been found to be satisfactory. The residue must be taken up in dilute nitric acid rather than hydrochloric to prevent precipitation of AgCl.

Instrumental Parameters (General)

1. Silver hollow cathode lamp
2. Wavelength: 328.1 nm
3. Type of burner: Boling
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of Flame: Oxidizing
7. Photomultiplier tube: 1P28

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Interference from high concentrations (1000 mg/l) of calcium, aluminum, potassium, magnesium, phosphate, sulfate, nitrate and silicate may be observed. The use of the nitrous oxide-acetylene flame will lessen this interference with some loss of sensitivity.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Silver nitrate standards are light sensitive. Dilutions of the stock should be discarded after use as concentrations below 10 mg/l are not stable over long periods of time.
3. The 338.2 nm wavelength may also be used. This has a relative sensitivity of 3.

THALLIUM

STORET NO:

(Standard Conditions) TOTAL : 01059

Optimum Concentration Range 1-20 mg/l using a wavelength of 276.8 nm

Sensitivity 0.2 mg/l

Detection Limit 0.05 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 1.303 grams of thallium nitrate, $TlNO_3$ (analytical reagent grade) in distilled water. Add 10 ml of concentrated nitric acid and dilute to 1 liter with distilled water. One ml equals 1 mg Tl (1000 mg/l).
2. Prepare dilutions of the stock thallium solution to be used as calibration standards at the time of analysis. Maintain an acid strength of 0.15% nitric acid in all calibration standards.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General)

1. Thallium hollow cathode lamp.
2. Wavelength: 276.8 nm
3. Type of burner: Boling
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of flame: Stoichiometric
7. Photomultiplier tube: R 106

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Interference from high concentrations (1000 mg/l) of calcium, aluminum, potassium, magnesium, phosphate, sulfate, nitrate, and silicate may be observed. The use of the nitrous oxide-acetylene flame will lessen this interference with some loss of sensitivity.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

TIN

STORET NO:
TOTAL : 01102

(Standard Conditions)

Optimum Concentration Range: 10-200 mg/l using a wavelength of 235.5 nm.

Sensitivity: 2 mg/l

Detection Limit: 0.4 mg/l

Preparation of Standard Solution:

1. Stock Solution: Dissolve 1.000 gram of tin metal (analytical reagent grade) in 100 ml of concentrated HCl and dilute to 1 liter with distilled water. One ml equals 1 mg Sn (1000 mg/l).
2. Prepare dilutions of the stock tin solution to be used as calibration standards at the time of analysis. Maintain an acid concentration of 10% HCl in all solutions.

Sample Preparation:

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes, 1971" (p 88, 4.1.3) has been found to be satisfactory.

Instrumental Parameters (General):

1. Tin hollow cathode lamp
2. Wavelength: 235.5 nm
3. Type of burner: Boling
4. Fuel: Acetylene
5. Oxidant: Air
6. Type of flame: Fuel rich
7. Photomultiplier tube: R 106

Interferences:

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Interference from high concentrations (1000 mg/l) of calcium, aluminum, potassium, magnesium, phosphate, sulfate, nitrate and silicate may be observed. The use of the nitrous oxide-acetylene flame will lessen this interference with some loss of sensitivity.

Notes:

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

TITANIUM

STORET NO:
(Standard Conditions) TOTAL : 01152

Optimum Concentration Range 2-100 mg/l using a wavelength of 364.3 nm

Sensitivity 1.0 mg/l

Detection Limit 0.3 mg/l

Preparation of Standard Solution

1. Stock Solution: Dissolve 4.008 grams of titanium sulfate ($Ti_2 (SO_4)_3$) in dilute HCl and make up to 1 liter with distilled water. One ml equals 1 mg Ti (1000 mg/l).
2. Potassium chloride solution: Dissolve 95g potassium chloride, KCl in distilled water and make up to 1 liter.
3. Prepare dilutions of the stock titanium solution to be used as calibration standards at the time of analysis. To each 100 ml of standard and sample alike, add 2 ml of potassium chloride solution.

Sample Preparation

1. The procedure for the determination of total metals as given in "Methods for Chemical Analysis of Water and Wastes", 1971 (p 88, 4.1.3) must be modified by the addition of 3 ml of concentrated sulfuric acid in addition to the nitric acid. This is necessary to keep any titanium that may be present in solution.

Instrumental Parameters (General)

1. Titanium hollow cathode lamp
2. Wavelength: 365.3 nm
3. Type of burner: Nitrous Oxide

4. Fuel: Acetylene
5. Oxidant: Nitrous Oxide
6. Type of flame: Fuel rich
7. Photomultiplier: 1P28

Interferences

1. The presence of high dissolved solids in the sample may result in an interference from non-atomic absorbance such as light scattering. If background correction is not available, a non-absorbing wavelength should be checked.
2. Titanium is easily ionized in the flame and potassium (1000 mg/l) must be added to standards and samples alike to control this effect.

Notes

1. Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition must be used.
2. Data to be entered into STORET must be reported as µg/l.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1973— 759-555/1154

CYANIDE, Total

STORET NO. 00720

1. Scope and Application

   1.1 This method is applicable to the determination of cyanide in surface waters, domestic and industrial wastes, and saline waters.

   1.2 The titration procedure using silver nitrate with p-dimethylamino-benzalrhodanine indicator is used for measuring concentrations of cyanide exceeding 1 mg/l (0.2 mg/200 ml of absorbing liquid).

   1.3 The colorimetric procedure is used for concentrations below 1 mg/l of cyanide and is sensitive to about .02 mg/l.

2. Summary of Method

   2.1 The cyanide as hydrocyanic acid (HCN) is released from metallic cyanide complex ions by means of a reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by volumetric titration or colorimetrically.

   2.2 In the colorimetric measurement the cyanide is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at a pH less than 8 without hydrolyzing to the cyanate. After the reaction is complete, the CNCl forms a red-blue dye on the addition of a pyridine-barbituric acid reagent. The absorbance is read at 578 nm. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards.

   2.3 The titrimetric measurement uses a standard solution of silver nitrate to titrate cyanide in the presence of a silver sensitive indicator.

3. Definitions

3.1 Cyanide is defined as cyanide ion and complex cyanides converted to hydrocyanic acid (HCN) by reaction in a reflux system of a mineral acid in the presence of cuprous ion.

4. Sample Handling and Preservation

4.1 The sample should be collected in plastic bottles of 1 liter or larger size. All bottles must be thoroughly cleansed and thoroughly rinsed to remove soluble material from containers.

4.2 Samples must be preserved with 2 ml of 10 N sodium hydroxide per liter of sample (pH $\geq$ 12) at the time of collection.

4.3 Samples should be analyzed as rapidly as possible after collection. If storage is required, the samples should be stored in a refrigerator or in an ice chest filled with water and ice to maintain temperature at 4°C.

4.4 Oxidizing agents such as chlorine decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch test paper (KI-starch paper); a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper. Then add an additional 0.6 gram of ascorbic acid for each liter of sample volume.

5. Interferences

5.1 Interferences are eliminated or reduced by using the distillation procedure described in Procedure (8.1 through 8.5).

5.2 Sulfides adversely affect the colorimetric and titration procedures. If a drop of the sample on lead acetate test

6. Apparatus

6.1 Reflux distillation apparatus such as shown in Figure 1 or Figure 2. The boiling flask should be of 1 liter size with inlet tube and provision for condenser. The gas absorber may be a Fisher-Milligan scrubber.

6.2 Microburet, 5.0 ml (for titration).

6.3 Spectrophotometer suitable for measurements at 578 nm with a 1.0 cm cell or larger.

7. Reagents

7.1 Sodium hydroxide solution. Dissolve 50 g of NaOH in distilled water, and dilute to a liter with distilled water.

7.2 Cadmium carbonate.

7.3 Ascorbic acid.

7.4 Cuprous Chloride Reagent - Weigh 20 g of finely powdered Cu_2Cl_2 into an 800-ml beaker. Wash twice, by decantation, with 250-ml portions of dilute sulfuric acid (H_2SO_4, 1 + 49) and then twice with water. Add about 250 ml of water and then hydrochloric acid (HCl, sp gr 1.19) in 1/2-ml portions until the salt dissolves (Note 1). Dilute to 1 liter with water and store in a tightly stoppered bottle containing a few lengths of pure copper wire or rod extending from the bottom to the mouth of the bottle (Note 2).

Note 1: The reagent should be clear; dark discoloration indicates the presence of cupric salts.

Note 2: If it is desired to use a reagent bottle of smaller volume, it should be kept completely filled and tightly stoppered. Refill it from the stock solution after each use.

paper indicates the presence of sulfides, treat 25 ml more of the stabilized sample (pH $\geq$12) than that required for the cyanide determination with powdered cadmium carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of cadmium and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

5.3 Fatty acids will distill and form soaps under the alkaline titration conditions, making the end point almost impossible to detect. Fatty acids are removed by extraction as suggested by Kruse and Mellon. Acidify the sample with acetic acid (1 + 9) to pH 6.0 to 7.0. (Caution--This operation must be performed in the hood and the sample left there until it can be made alkaline again after the extraction has been performed.) Extract with iso-octane, hexane, or chloroform (preference in order named) with a solvent volume equal to 20 percent of the sample volume. One extraction is usually adequate to reduce the fatty acids below the interference level. Avoid multiple extractions or a long contact time at low pH in order to keep the loss of HCN at a minimum. When the extraction is completed, immediately raise the pH of the sample to above 12 with NaOH solution.

7.5 Sulfuric acid, concentrated.

7.6 Sodium dihydrogenphosphate, 1 M. Dissolve 138 g of $NaH_2PO_4.H_2O$ in one liter of distilled water. Refrigerate this solution.

7.7 Stock cyanide solution. Dissolve 2.51 g of KCN and 2 g KOH in one liter of distilled water. Standardize with 0.0192 N $AgNO_3$. Dilute to appropriate concentration so that 1 ml = 1 mg CN^-.

7.8 Standard cyanide solution, intermediate. Dilute 10 ml of stock (1 ml = 1 mg CN) to a liter of distilled water (1 ml = 10 µg).

7.9 Standard cyanide solution. Prepare fresh daily by diluting 100 ml of intermediate cyanide solution to a liter of distilled water and store in a glass stoppered bottle. One ml = 1.0 µg CN (1.0 mg/l).

7.10 Standard silver nitrate solution, 0.0192 N. Prepare by crushing approximately 5 g $AgNO_3$ crystals and drying to constant weight at 40°C. Weigh out 3.2647 g of dried $AgNO_3$, dissolve in water, and dilute to 1.0 liter (1 ml = 1 mg CN).

7.11 Rhodanine indicator. Dissolve 20 mg of p-dimethylamino-benzal-rhodanine in 100 ml of acetone.

7.12 Chloramine T solution. Dissolve 1.0 g of white water soluble Chloramine T in 100 ml of distilled water and refrigerate until ready to use. Prepare fresh weekly.

7.13 Pyridine-Barbituric Acid Reagent. Place 15 g of barbituric acid in a 250-ml volumetric flask and add just enough water to wash the sides of the flask and wet the barbituric acid. Add 75 ml of pyridine and mix. Add 15 ml of HCl (sp gr 1.19), mix, and cool to room temperature. Dilute to 250 ml with water and mix.

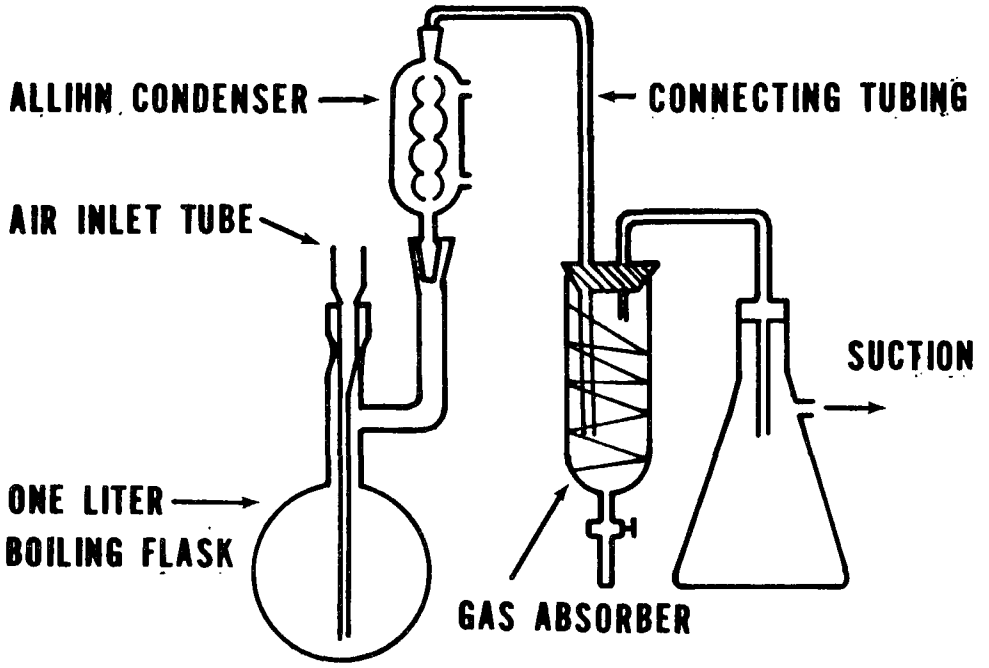


FIGURE 1

CYANIDE DISTILLATION APPARATUS

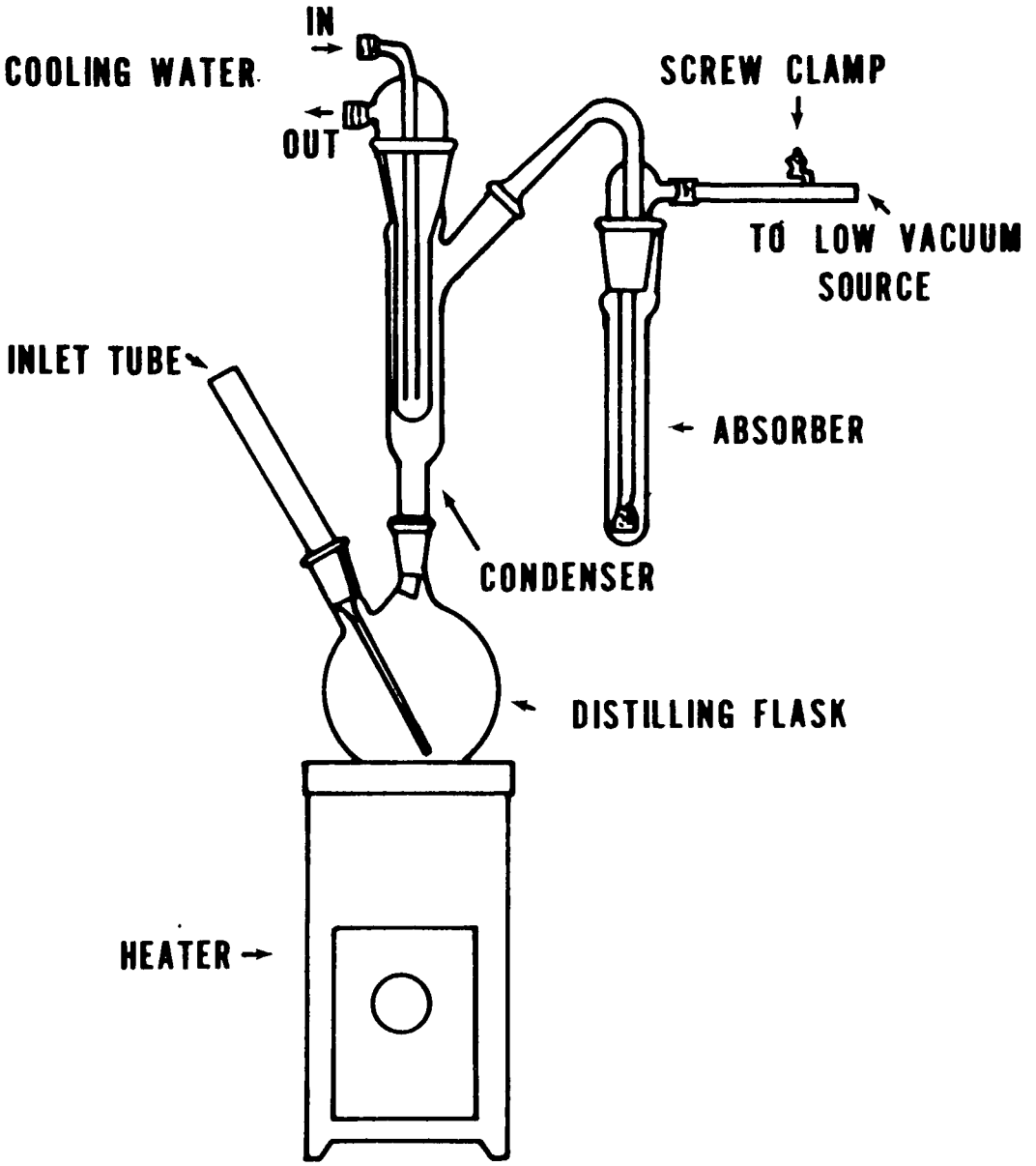


FIGURE 2

CYANIDE DISTILLATION APPARATUS

8. Procedure

8.1 Place 500 ml of sample, or an aliquot diluted to 500 ml in the 1-liter boiling flask. Add 50 ml of sodium hydroxide (7.1) to the absorbing tube and dilute if necessary with distilled water to obtain an adequate depth of liquid in the absorber. Connect the boiling flask, condenser, absorber and trap in the train.

8.2 Start a slow stream of air entering the boiling flask by adjusting the vacuum source. Adjust the vacuum so that approximately one bubble of air per second enters the boiling flask through the air inlet tube. (Caution: The bubble rate will not remain constant after the reagents have been added and while heat is being applied to the flask. It will be necessary to readjust the air rate occasionally to prevent the solution in the boiling flask from backing up into the air inlet tube).

8.3 Slowly add 25 ml concentrated sulfuric acid (7.5) through the air inlet tube. Rinse the tube with distilled water and allow the airflow to mix the flask contents for 3 min. Pour 10 ml of Cu_2Cl_2 reagent (7.4) into the air inlet and wash down with a stream of water.

8.4 Heat the solution to boiling, taking care to prevent the solution from backing up into the overflowing from the air inlet tube. Reflux for one hour. Turn off heat and continue the airflow for at least 15 minutes. After cooling the boiling flask, disconnect absorber and close off the vacuum source.

8.5 Drain the solution from the absorber into a 250 ml volumetric flask and bring up to volume with distilled water washings from the absorber tube.

8.6 Withdraw 50 ml of the solution from the volumetric flask and transfer to a 100-ml volumetric flask. Add 15 ml of sodium phosphate solution (7.6) and 2.0 ml of Chloramine T solution (7.12) and mix. Immediately add 5.0 ml pyridine-barbituric acid solution (7.13), mix and bring to mark with distilled water and mix again. Allow 8 minutes for color development.

8.7 Read absorbance at 578 nm in a 1.0 cm cell within 15 minutes.

8.8 Prepare a series of standards by diluting suitable volumes of standard solution to 500.0 ml with distilled water as follows:

ml of Standard Solution (1.0 ml = 1 µg CN)	Conc., When Diluted to 500 ml, mg/l CN
0 (Blank)	0
5.0	0.01
10.0	0.02
20.0	0.04
50.0	0.10
100.0	0.20
150.0	0.30
200.0	0.40

8.8.1 Standards must be treated in the same manner as the samples, as outlined in 8.1 through 8.7 above.

8.8.2 Prepare a standard curve by plotting absorbance of standards vs. cyanide concentrations.

8.8.3 Subsequently, at least two standards (a high and a low) should be treated as in 8.8.1 to verify standard curve. If results are not comparable (±10%), a complete new standard curve must be prepared.

8.8.4 To check the efficiency of the sample distillation, add an increment of cyanide from either the intermediate standard (7.8) or the working standard (7.9) to insure a level of 10 µg/l or a

significant increase in absorbance value. Proceed with the analysis as in Procedure (8.8.1) using the same flask and system from which the previous sample was just distilled.

8.9 Alternatively, if the sample contains more than 1 mg of CN^- transfer the distillate, or a suitable aliquot diluted to 250 ml, to a 500-ml Erlenmeyer flask. Add 10-12 drops of the benzalrhodamine indicator.

8.10 Titrate with standard silver nitrate to the first change in color from yellow to brownish-pink. Titrate a distilled water blank using the same amount of sodium hydroxide and indicator as in the sample.

8.11 The analyst should familiarize himself with the end point of the titration and the amount of indicator to be used before actually titrating the samples. A 5 or 10 ml microburet may be conveniently used to obtain a more precise titration.

9. Calculation

9.1 Using the colorimetric procedure, calculate concentration of CN, mg/l, directly from prepared standard curve.

9.2 Using the titrimetric procedure, calculate concentration of CN as follows:

$$\text{CN, mg/l} = \frac{(A-B) \times 1000}{\text{Vol. of original sample}} \times \frac{250}{\text{Vol. of aliquot titrated}}$$

where:

A = volume of $AgNO_3$ for titration of sample.

B = volume of $AgNO_3$ for titration of blank.

References

1. Bark, L. S., and Higson, H. G. Investigation of reagents for the colorimetric determination of small amounts of cyanide. Talanta, 2:471-479 (1964).

2. Elly, C. T. Recovery of cyanides by modified Serfass distillation. Journal Water Pollution Control Federation, 40:848-856 (1968).

☆ U.S. GOVERNMENT PRINTING OFFICE 1973— [illegible]

CHEMICAL ANALYSIS FOR DEMAND, NUTRIENT AND OIL AND GREASE

By

Ho Young
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

The primary purpose of the chemistry lectures is to describe briefly the analytical methods in recent or current use in EPA laboratories for determining oxygen demand, nutrients, oil and grease, metals and pesticides. Secondarily, they are to point out the advantages and the limitations of these methods.

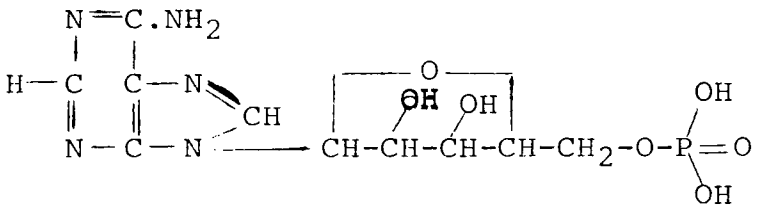
The first three parameters measure the various commonly existing materials present in water and the waste discharges.

ORGANIC CONTENT OF WASTES

I. Definition of organic materials.
II. Properties of organic materials.
    A. Organic compounds are usually combustible.
    B. Organic compounds, generally, have lower melting and boiling points.
    C. Organic compounds are usually less soluble in water.
    D. Reactions of organic compounds are usually molecular rather than ionic.

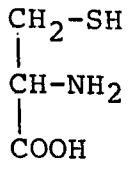
$$2C_2H_6 + 7O_2 \longrightarrow 4CO_2 + 6H_2O$$

    E. Molecules are usually larger and heavier than those in inorganic substances.
    F. Most organic compounds can serve as a source of food for microorganisms.
III. Types of organic substances in wastes.
    A. Simple naturally-occurring organic compounds.
        1. Hydrocarbons
        2. Carbohydrates
    B. Complex naturally-occurring organic compounds.
        1. Organic nitrogen compounds--amino acids, protein.
        2. Organic phosphate compounds--nucleic acid, ADP, ATP, etc.



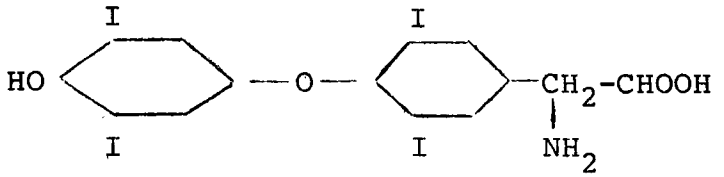
adenine ribose phosphate

3. Organic sulfate compounds--amino acids with a sulfur group, e.g. cystine, cysteine, mathionine.



cysteine

4. Organic halogen compounds--thyroxine, thyroid hormone.



thyroxine

C. Synthetic organic compounds.
   1. Chlorinated organic compounds--polychlorinated biphenyls
   2. Pesticides--organochlorinated Pesticides such as aldrin, dieldrin, DDT; o-aryl carbamate pesticides; organophosphorus pesticides; and triazine pesticides.
   3. Chlorinated phenoxy acid herbicides.

IV. Analytical methods for organic substances in wastes.
   A. Direct measurements
      1. Total organic phosphate and orthophosphate.
      2. Total organic carbon.
      3. Total Kjeldahl nitrogen, ammonia, and nitrates.
   B. Indirect measurements.
      1. Biochemical oxygen demand.
      2. Chemical oxygen demand.

Prepared by Ho Lee Young, Ph.D.
Chief, Chemistry Section
Laboratory Support Branch
EPA, Region IX
March 1, 1974

ANALYSIS OF ORGANIC COMPOUNDS

I. Oxygen Demands: It is a determination of the decrease of dissolved oxygen in receiving water when waste is discharged into water.

A. Objective: This is an indirect method to measure the oxidizable organics of the waste.

B. Measurement Techniques

1. Oxygen Probe
2. Winkler - Azide Method

C. Biological Oxygen Demands (BOD).

1. Principle: It is an estimate of the biodegradeable organic materials of the sample by establishing the amount of decrease of dissolved oxygen. The BOD test gives an indication of the amount of oxygen needed to stabilize or biologically oxidize the waste.

2. Types of biodegradable organic materials measured in BOD test.

a. Organic Carbon: carbohydrates (common sugars and their metabolic by-products) and hydrocarbons.

b. Nitrogenous compounds: amino acids, nitrates, ammonia and some complex nitrogenous compounds such as nucleic acid, nucleotides and nucleosides.

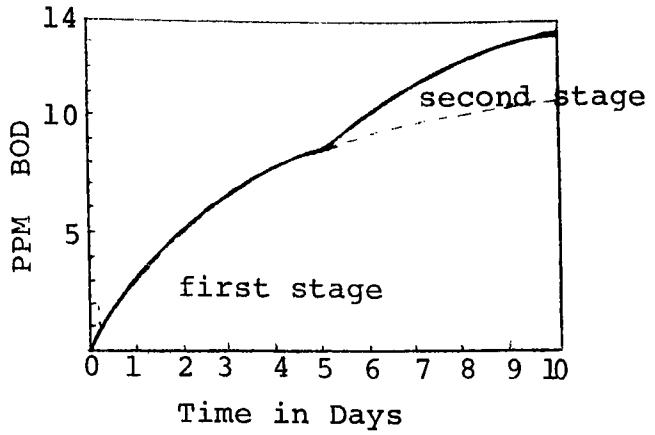
3. Types of BOD measurement

a. Dynamic measurement: to measure the change or the rate of change in oxygen utilization with respect to time.

1. Manometric method: Warburg Apparatus
2. Electrolysis BOD devices: Hach Apparatus

b. Static measurement: to measure the amount of oxygen used at a fixed time interval.

1. Ultimate BOD: to measure the amount of oxygen required to oxidize the entire amount of the biodegradeable materials.



First Stage BOD - mainly oxidation of carbon compounds.
Second stage BOD - low rate oxidation of most resistant compounds and/or nitrogenous compounds.

2. BOD_5: An emperical bioassay type procedure. It, in general, with the corrected dilution, measures the oxygen consumption from oxidation of carbon sources.

   It measures the dissolved oxygen consumed by microorganisms while assimilating and oxidizing the organic matter present during incubation. The incubation period is five days. Incubation conditions are 20°C in the dark, and pH near neutrality. Seeds used are common sewage bacteria or commercial septic activator.

4. Advantage and limitations

   a. Advantage: it measures only the organic compounds which are oxidized by the microorganisms, mainly bacteria.

   b. Limitations

      1. The difficulty in obtaining consistent and repetitive values - variations in the time lag between sampling and results of analysis.

      2. The actual environmental conditions of temperature, biological population and seed acclimation, water movement, sunlight and oxygen concentration cannot be accurately reproduced in the laboratory.

3. Results obtained in the laboratory may or may not represent the oxygen demands at the effluent site.

4. Accumulation of CO_2 influences the test results.

5. Lack of seed acclimation results in erroneously low readings.

6. Sea water interferes because of salinity differences due to dilution.

D. Chemical Oxygen Demand (COD)

1. Principle: It is an estimate of that proportion of the sample matter which is susceptible to oxidation by a strongchemical oxidant.

2. Types of substances oxidized by dichromate in 50% sulfuric acid.

a. Sugars, branched and straight chain aliphatics and substituted benzene rings.

b. Straight-chain acids, alcohols and amino acids can be completely oxidized in the presence of the silver sulfate catalyst.

Benzene, pyridine and toluene are not oxidized by this method.

3. Procedures

4. Advantages and limitations

a. Advantages as compared to BOD

1. Time, manipulation, and equipment costs are lower.

2. COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3. COD test conditions can be standardized more readily to give more precise results.

4. COD results are available in few hours.

5. The COD results plus the oxygen equivalent for ammonia and organic nitorgen is a good estimate of the ultimate BOD for many municipal wastewaters.

b. Limitations

1. Certain inorganic substances, such as sulfides, sulfites, thiosulfates, nitrites and ferrous iron are oxidized by dichromate, creating an inorganic COD, which is misleading when estimating the organic content of the wastewater.

2. The COD test may not include some volatile organics such as acetic acid and ammonia.

3. Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4. Because of chloride interference, it is not advisable to expect precise COD results on saline water.

5. Requires a large quantity of mercuric sulfate which is a pollutant.

II. Total Organic Carbon (TOC)

A. Principle: all carbon atoms of organic molecules are oxidized to CO_2 at high temperature; the amount of CO_2 produced is measured by an infra-red analyzer.

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

B. Methods

1. Direct injection: carbon atoms are combusted at 950° C.

2. Indirect digestion: carbon atoms are oxidized in acid at 166° C in the presence of pure oxygen.

C. Advantages and Limitations

1. Advantages

a. Speed, direct injection method takes 2 minutes.

b. To measure the total carbon of all forms.

2. Limitations: carbonate and bicarbonate interfers with the analysis.

III. Relationships between BOD, COD and TOC's

BOD is not the most useful test of waste load because of the long incubation time required to obtain a meaningful result. It is, therefore, important to develop a correlation between BOD, COD and TOC.

Table 1
Comparison of BOD, COD and TOC Tests

	BOD	COD	TOC
Test temp °C	20	145	950° or 166° with pressure
Reaction time	5 days	2 hrs.	minute or hours
Oxidation system	Biol. prod. Enz. Oxidn.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	oxygen, atmosphere, catalyzed
Measurement Variables	Dissolved oxygen compound, environment biota, time numbers, metabolic acceptability etc.	Chemical oxidation susceptibility of the test sample to the specified oxidation	Infra-red CO_2 comparable to theoretical for carbon only.
Equipment	Bottles Incubator	heater glassware	TOC Analyzer
Cost	$150	$500	$8000

Table 2
COD-TOC and BOD_5-TOC Relationships

Substance	COD/TOC	BOD_5/TOC
Acetone	2.44 (3.56)*	
Ethanol	3.35 (4.00)	
Phenol	2.96 (3.12)	
Salicylic	2.83 (2.86)	
Methanol	3.89 (4.00)	
Benzoic Acid	2.90 (2.86)	
Sucrose	2.44 (2.67)	
Benzene	0.84 (3.34)	
Pyridine	nil (3.33)	

Waste	Raw	Effluent	Raw	Effluent
Domestic	4.15	2.20	1.62	0.47
Chemical	3.54	2.29		
Refinery-Chemical	5.40	2.15	2.75	0.43
Petrochemical	2.70	1.85		

*Values in parenthesis are the theoretical values.

IV. Nutrients: These include nitrogenous and phosphorus compounds.

A. Nitrogenous compounds: ammonia (NH_3) nitrite (NO_2), nitrate (NO_3) and total organic nitrogen (TKN)

1. Procedures: Table 3

	NH_3	NO_2	NO_3	TKN
Sample Preparation	distilled from alkaline sol, absorbed in borate buffer	formation of diazonium compound with diazotatin of sulfanilanide, coupled with N-(1-naphtyl) -ethylene diamine-reddish purple	reaction with brucine sulfate in H_2SO_4	acid digestion, distilled and absorbed in borate buffer
Detection	1. colorimetric method by Nesslarization 400-425nm 2. titration with acid	spectrophotometric, at 540 nm.	spectrophotometric at 410nm.	1. colorimetric method by Nesslerization 400-425 nm. 2. titration with acid.
Range	0.05-1 mg/l 1-25 mg/l	0.05-1 mg/l	0.1-2 mg/l	0.05-1 mg/l 1-25 mg/l
Interferences	Cyanates alcohols aldehydes and ketones	strong oxidizing or reducing agents	strong oxidizing and reducing agents Fe^{+++}, Fe^{++} Mn^{++}, Cl^- organic matter	

2. Other analytical methods usable for samples containing high salt concentration - to be discussed.

V. Phosphorate Compounds: Phosphorus is usually present as orthophosphate, polyphosphate, and organically bound phosphorus.

A. EPA Spectrophotometric Method (p. 252).

1. Principle: It is an analysis of the total phosphorus by the formation of antimony-phosphomolybdate complex.

2. Methods

   a. Polyphosphates are rapidly hydrolized into orthophosphate in boiling water at low pH.

   b. Organic forms of phosphorus are converted to orthophosphates by wet oxidation.

   c. Orthophosphate reacts with ammonium molybdate and potassium antimonyl tartrate in acid medium to form antimony - phosphomolybdate complex.

   d. The complex is reduced to an intensely blue-colored complex by ascorbic acid.

   e. The blue color which is proportional to the concentration of phosphorus is measured at 880 nm.

3. Interference

   a. Cl^- concentration below 50 mg Cl/l interferes.

   b. High iron concentration causes precipitation of phosphorus.

   c. Arsenic at sea water level does not interfere.

B. Phosphate classification

Table 4

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique[1]	Incidental P Included[2]
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

VI. Oil and Grease

A. Sources

1. Industrial waste: petroleum product, lubrication oil.

2. Decomposition of planktons and higher forms of aquatic life.

B. Principle: dissolved, emulsified or adsorbed oil or grease is extracted by intimate contact with various organic solvents.

C. Types of extractions: liquid-liquid and soxhlet extractions as discussed below:

Table 5. Summary of oil and grease analysis

Sample	Aqueous	Sediment tissues
Sample Preservation	acidification	Freezing
Solvent	Trichlorotrifluoro-ethane (Freon)	Hexane
Sample Preparation	Acidified - H_2SO_4	Acidified-HCl to pH 2.0 dehydration with magnesium sulfate monohydrate
Extraction temp	Room temperature	70°C
Extraction time	Two minutes vigorously. Shaking in separatory funnel.	4 hrs. (80 cycles)
Extractable Material	Oils, lubrication oil, fats	soaps, fats, waxes, and oil
Interferences	Evaporation of low boiling oils, kerosene	elementary S, organic dye, and oxidation of oil.

VII. References

1. Standard Methods for the Examination of Water and Wastewater, 13th Edition, American Public Health Association, 1015 18th Street, N.W. Washington, D.C.

2. Methods for Chemical Analysis of Water and Wastes, EPA, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio, 1971.

3. J.D.H. Strickland and T.R. Parsons. A Practical Handbook of Seawater Analysis, Fisheries Research Board of Canada, Ottawa, 1968.

Prepared by
Ho Lee Young, Ph.D.
Chief, Chemistry Section
Laboratory Support Branch
EPA, Region IX

March 1, 1974

AMOUNT OF DISSOLVED OXYGEN IN WATER AT DIFFERENT TEMPERATURES WHEN EXPOSED TO AN ATMOSPHERE CONTAINING 20.9 PER CENT OF OXYGEN UNDER A PRESSURE OF 760 MM. INCLUDING PRESSURE OF WATER VAPOR*

Temp. °C.	*Parts per Million*	*Cc. per liter (at 0° C. and 760 mm.)*	*Temp. °C.*	*Parts per Million*	*Cc. per liter (at 0° C. and 760 mm.)*
0	14.62	10.23	16	9.95	6.96
1	14.23	9.96	17	9.74	6.82
2	13.84	9.68	18	9.54	6.68
3	13.48	9.43	19	9.35	6.54
4	13.13	9.19	20	9.17	6.42
5	12.80	8.96	21	8.99	6.29
6	12.48	8.73	22	8.83	6.18
7	12.17	8.52	23	8.68	6.07
8	11.87	8.31	24	8.53	5.97
9	11.59	8.11	25	8.38	5.86
10	11.33	7.93	26	8.22	5.75
11	11.08	7.75	27	8.07	5.65
12	10.83	7.58	28	7.92	5.54
13	10.60	7.42	29	7.77	5.44
14	10.37	7.26	30	7.63	5.34
15	10.15	7.10			

D. O. MEASUREMENT WITH OXYGEN PROBE

1. Oxygen probe with the stirrer attached should be kept in a moistened BOD bottle at all times.
2. Meter on off position.
3. Switch to check position - point should read "check"
4. 0 - 15 range, warm up for 10 minutes.
5. Remove probe gently and carefully. If the probe sticks in the bottle's neck, turn the probe slowly to free it.
6. Cover and shake the BOD bottle vigorously for 15 seconds.
7. Remove the stirring bar (at the bottom) from the probe.
8. Wipe the probe very carefully to remove moisture.
9. Shake the bottle again.
10. Insert the probe into the bottle.
11. Set function switch at temp, note the temperature.
12. Read the solubility of oxygen in fresh water at that temperature from Table I (on top of the dissolved Oxygen meter) for example at 23°C = 8.7 mg/liter.
13. Turn function switch to 0 - 15 range.
14. Adjust calibration knob until the meter reads the proper dissolved Oxygen at that temperature. (e.g. 8.7)
15. Repeat steps 8 to 14.
16. Turn the function switch to temperature or check.
17. Assemble the stirring bar assembly to the probe. Probe is ready to be used for measuring D.O. in sample.
18. Insert Oxygen probe into the sample bottle very carefully. If the BOD bottle is too small, do not force the probe in, just discard the sample.
19. Place the sample bottle on a magnetic stirrer.
20. Be sure no air bubble(s) trapped on the surface of the membrane (look at the bottom of the probe through the sample bottle). Remove air bubble by raising the probe above the water surface and insert the probe again.
21. Turn on the magnetic stirrer.
22. Turn function switch to 0 - 15 range.
23. Allow sufficient time for the probe to equilibriate with the sample - up to 2 minutes.

24. Record the dissolved oxygen reading e.g. 2.1 mg/l.
25. Turn the function switch to temp.
26. Turn probe around to loosen the water seal, then remove the probe.
27. When the measurement is finished, return the probe into the moistened BOD bottle.
28. Turn off the meter.
29. Turn off the magnetic stirrer.

Winkler - Azide Method for Dissolved Oxygen

I. Reagents

1. Alkali - iodide - azide reagent (NaOH - NaI - NaN_3 or KOH - KI - NaN_3).
2. Manganese sulfate solution ($MnSO_4$).
3. 0.0375 N Potassium biiodate standard [$KH(IO_3)2$].
4. Potassium Iodide (KI crystals).
5. Sodium thiosulfate titrant ($Na_2S_2O_3$).
6. Starch Solution.
7. Sulfuric acid (concentrated H_2SO_4).
8. 10% Sulfuric acid (10% H_2SO_4).

II. Standardization of $Na_2S_2O_3$ with Primary Standard, 0.0375 N potassium biiodate [$KH(IO_3)_2$].

1. Dissolve 2 g of KI crystals (two level scoops full) with 100 - 150 ml of distilled water in a wide-mouth 500 ml Erlenmeyer flask.
2. Add 10 ml of 10% H_2SO_4 into the KI solution and mix well.
3. Add 20 ml of 0.0375 N KH $(IO_3)_2$ and mix well.
4. Place in dark for five minutes.
5. Fill a 25 ml buret with $Na_2S_2O_3$.
6. Bring KI - H_2SO_4 - $KH(IO_3)_2$ mixture to a total volume of approximately 300 ml.
7. With the magnetic stirrer set at moderate speed, titrate the mixture with $Na_2S_2O_3$ until the color of the solution turns to a pale straw color.
8. Add 1 ml of starch solution (2 droppers full) to the the mixture, and mix well.
9. Continue the titration until the mixture turns colorless.

10. Record the amount of $Na_2S_2O_3$ used for titration.

11. Compute the normality of $Na_2S_2O_3$.

$$\text{Normality of } Na_2S_2O_3 = \frac{\text{ml of } KH(IO_3)_2 \times \text{Normality of } KH(IO_3)_2}{\text{ml of } Na_2S_2O_3}$$

III. Titration of dissolved oxygen of a sample.

1. Fill the BOD bottle up to the top with sample. Note: for a predicted BOD 5 mg/l, pipet a proper amount of sample* into a BOD bottle and fill the bottle with oxygenated dilution water (Dilution water: Distilled water aerated overnight containing 1 ml $CaCl_2$ solution, 1 ml $FeCl_3$ solution, 1 ml $MgSO_4$ solution and 1 ml phosphate buffer per liter).

2. Add 2 ml of $MnSO_4$ and 2 ml of alkali - iodide - azide reagent. Make sure the tip of the pipet is immersed well below the surface of the sample to prevent formation of bubbles.

3. Immediately stopper the bottle, with care to exclude air bubbles.

4. Mix the solution well by inverting the bottle at least five times.

5. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, mix the solution again.

6. When settling has produced at least 100 ml of clear supernatant on the upper portion, carefully remove the stopper and immediately add 2 ml of concentrated H_2SO_4 to the mixture.

7. Stopper the bottle and invert the bottle until the iodide is uniformly distributed throughout the bottle.

8. Transfer the entire sample into a 500 ml wide-mouth Erlenmeyer flask.

9. Titrate the sample with the standardized $Na_2S_2O_3$ solution as described in items 7-9 of part II.

10. Record the quantity of $Na_2S_2O_3$ used for the titration.

11. Compute the dissolved oxygen in the sample.

$$\text{DO, mg/l} = \text{ml of } Na_2S_2O_3 \times \frac{\text{Normality of } Na_2S_2O_3}{\text{Normality of } KH(IO_3)_2}$$

$$= \text{ml of } Na_2S_2O_3 \times \frac{\text{Normality of } Na_2S_2O_3}{0.0375 \text{ N}}$$

REFERENCE: Dissolved Oxygen: Methods For Chemical Analysis Of Wastes, 1971, Environmental Protection Agency, pp 53-59

BOD: IBID pp 15-16

FOOTNOTE

*Sample size for a BOD_5 5 mg/l when using 300 ml BOD bottle and dilution water (The initial dissolved oxygen of the mixture should be 7.0 mg/l).

Sample Size	mg/l BOD Range Covered
1 ml	300 - 1800 mg/l
2 ml	150 - 900 mg/l
3 ml	100 - 600 mg/l
4 ml	75 - 450 mg/l
5 ml	60 - 360 mg/l
6 ml	50 - 300 mg/l
7 ml	43 - 257 mg/l
8 ml	38 - 225 mg/l
9 ml	33 - 200 mg/l
10 ml	30 - 180 mg/l
15 ml	20 - 120 mg/l
20 ml	15 - 90 mg/l
25 ml	12 - 72 mg/l
30 ml	10 - 60 mg/l
40 ml	7.5 - 45 mg/l
50 ml	6 - 36 mg/l
75 ml	4 - 24 mg/l
100 ml	3 - 18 mg/l
150 ml	2 - 12 mg/l
200 ml	1.5 - 9 mg/l
300 ml	1 - 6 mg/l

Definition of Terms Used in EPA Methods

Range of Applicability: This is intended to state the upper and lower concentration (or other appropriate characteristics of the parameter) for which the method is applicable. When only an upper or lower limit is given in the reference source, that information is entered. When non-quantitative information is given in the reference source, that information may also be entered under this heading.

Sensitivity: In general, sensitivity is used synonymously with "Detection Limit", to indicate the lowest concentration of a pollutant (or lowest value of some other parameters) that a given method can consistently measure. In a very few cases, the source reference or reviewer distinguishes between "Detection Limit" (as the lowest measurable value) and "Sensitivity" (as the magnitude of signal needed to obtain a reliable measurement, taking into account the noise level of the measurement system). When this distinction is made in the reference source, it is reflected in the Method Summary.

Sensitivity, in either of the senses discussed above, may be considered to be either a statistical characteristic or a limitation of the method. Sensitivity information was available for a relatively small proportion of the methods summarized. The "sensitivity" heading is not included on the Method Summaries when data are not available. When sensitivity information is reported, it is usually under the category of "Limitations".

Accuracy and Precision: There is considerable diversity in the use of these terms among the several reference sources from which the Method Summaries were derived. Rather than impose rigorous statistical definitions of these and related terms, the compilers of this compendium chose to accept the statistical characteristics of the method as stated in the reference source, and to fit this information as well as possible under the headings Accuracy and Precision, as these terms were used more or less consistently in most of the EPA source documents. In these sources, the implied approximate definitions are as follows:

"Accuracy" -- the average of the deviations of a set of replicate measurements of a given variable from the "known" value of that variable.

"Relative accuracy" (or "relative error", or "bias") -- the difference between average value of a set of replicate measurements and the "known" value of the variable expressed as a proportion or percentage of the known value.

"Precision" -- either the standard deviation $\left[\left(\frac{\sum(Xi - \bar{x})^2}{n-1}\right)^{1/2}\right]$ or the standard error of the mean $\left[\left(\frac{\sum(Xi - \bar{x})^2}{n(n-1)}\right)^{1/2}\right]$ of a set of n replicate measurements (Xi) of a given variable.

"Relative precision" (or "relative standard deviation", or "coefficient of variation") -- the standard deviation of a set of replicate measurements, expressed as a proportion or percent of the average value of the set.

DEFINITIONS, CONVERSION FACTORS, AND EQUIVALENTS

% = parts per 100 parts
1 part per 100 parts = 1%
1 pound in 100 pounds = 1%

ppm = parts per million parts
1 pound per million pounds = 1 ppm
1 gram per million grams = 1 ppm
1 pound (English) = 453.6 grams (metric)
1 milligram per 1000 grams = 1 ppm
1 milligram = .001 gram
1 microgram per gram = 1 ppm
1 microgram = .000001 gram

ml/l = milliliters per liter
1 liter = 1000 milliliters (ml.)
1 milliliter (ml) = .001 liters (l)
1 liter = 1.057 quarts
1 quart (U.S.-Liquid) = 0.946 liters (metric-liquid)
1 ml = 1.000027 cm^3

mg/l = milligrams per liter = ppm
1 milligram = .001 gram (weight)
1 liter = 1000 grams (weight of water at standard conditions)
1 milliliter = 1 ml (volume) or .001 liters
1 milligram = 1 mg (weight) or .001 grams
1 milliliter of water weighs 1 gram (at standard conditions)

LENGTH (cont.)

1 inch	= 25.4 mm.
	= 2.54 cm.
1 foot	= 12 in.
	= 30.48 cm.
	= 0.3048 m.
1 yard	= 36 in. (3 feet)
	= 91.44 cm.
	= .9144 meters
1 fathom	= 6 ft.
1 league (land)	= 5280 yd.
	= 3 mi.
	= 4.828 km.
1 rod	= 16.5 ft.
	= 5.09210 meters
1 chain	= 66 feet

1 micron	= 1 X 10^{-3} mm.
1 mm	= 0.03937 in.
	= 0.1 cm.
1 centimeter	= 0.3937 in.
	= 10 mm.
1 meter	= 39.37 in.
	= 1.0936 yds.
	= 1000 mm.
	= 1 X 10^{-3} km.

AREA

1 sq. inch	= 6.452 $cm.^2$
1 sq. foot	= 144 $in.^2$
	= 929.0 $cm.^2$
1 sq. yard	= 9 $ft.^2$
	= 0.8361 $m.^2$
1 acre	= 43,560 $ft.^2$
	= 4046.9 $m.^2$
	= 0.4047 hectare
1 sq. mile	= 640 acres
	= 258.99 hectares
	= 2.590 $km.^2$
	= 1 section (of land)
1 sq. centimeter	= 0.1550 $in.^2$
	= 1 X 10^{-4} hectare meters^2 (1X10^{-8} ha)
1 hectare	= 2.471 acres
	= 107,640 $ft.^2$
	= 1 X 10^4 $m.^2$
	= 0.01 $km.^2$
	= 100 X 100 meters
1 sq. kilometer	= 247.1 acres
	= 0.3861 sq. miles
	= 1 X 10^6 $m.^2$
	= 100 hectares
	= 1000 X 1000 meters

VOLUME

1 cubic inch	= 16.39 cm^3
1 cubic foot	= 7.481 U.S. gallons
	= 1728 cu. inches
	= 28.32 liters
	= .0283 m^3
1 cubic yard	= 27 cubic feet
	= 0.7645 m^3
1 ounce (fluid)	= 29.57 ml.
1 pint	= 0.50 quarts
	= 0.125 gallons
	= 0.473 liters
1 quart	= 0.25 gallons
	= 946.25 milliliters
	= .946 liters
1 gallon	= 0.1337 cubic feet
	= 231 cubic inches
	= 3.785 liters
	= 0.83267 Imperial gallons
	= 8.337 lbs. water @ 62°F (8.345 lbs. @4°C)
1 cubic foot	= 7.481 gallons
	= 62.37 pounds
	= 28.32 liters
	= .0283 m^3
1 acre inch	= 3630 cubic feet
	= 27,150 gallons
	= 226000 pounds
	= 102.8 cubic meters
1 acre foot	= 43,560 cubic feet
	= 325,900 gallons
	= 2.716 million pounds
	= 1233.4 m^3
	= 12.173 ha cm
1 hectare centimeter	= .9728 acre in.
1 cubic centimeter	= 0.06102 cubic inches
	= 0.99997 milliliters
1 milliliter	= 1.000027 cm^3
	= .001 liters
1 liter	= 1.05680 quarts
	= 0.2642 gallons
	= 1000 milliliters
	= 1000.027 cm^3
	= .99997X10^{-3} m3
	= .03531 cubic feet
	= .001 m^3
	= .2201 Imperial gallon
1 cubic meter	= 264.2 gallons
	= 35.32 cubic feet
	= 1.308 cubic yards
	= 1000 liters
1000 cubic meters	= 35320 cubic feet
	= 0.81084 acre feet
	= 10 ha cm (.1 ha mm = 1 m^3 = 1 metric ton)
1000 cubic meters/hectare	= 0.32814 acre feet/acre
	= 10 ha cm/ha
1 cubic foot/sec.	= 1.98 acre feet/day
1 million gallons/day	= 3.069 acre feet/day
1 inch of rain	= 27,150 gallons/acre
barrel (oil)	= 42 gallons

FLOW RATE

1 gallon per minute	= .002228 cfs
	= .05304 ac. inches/day
	= 13860 $inch^3$/hr.
	= 96.25 $ft.^2$ covered 1" deep in 1 hr
	= 0.06308 lit/sec.
1 million gallons per day	= 694 gpm
	= 1.55 cfs
	= 3.07 AF/day
	= 43.7 l./sec.
1 cubic foot per second (cfs) - second foot, or CUSEC	= 448.83 U.S. gallons per minute
	= 0.99 acre inches/hr.
	= 1.98 AF/day
	= 28.32 l./sec.(1699.3 lit/min.)
	= .02832 cubic meters/sec.
1 acre-foot per day	= 226 gpm
	= 14.2 l./sec.
1 liter per second	= 15.852 gallons/min.
	= 0.0353 cfs
	= .03495 acre inches/hr.
	= 3.6 cubic meters/hour
	= 0.36 mm ha/hr.
1 cubic meter per second (CUMEC)	= 1.58 X 10^4 gpm
	= 35.314 cfs
	= 1000 l./sec.
1 cubic meter per hour	= 0.278 liters/sec.
	= 4.403 gallons/min.
miners inch (N. Calif)	= 1/40 cfs
	= 11.25 gpm
	= 0.6 ac. inch/day
miners inch (S. Calif.)	= 1/50 cfs
	= 9.0 gpm
	= 0.48 ac. inch/day

WEIGHT

1 grain	= 0.0648 gms
1 ounce	= 0.0625 lbs
	= 28.3495 gms
1 pound	= 453.5924 gms
	= 0.4536 kg.
1 short ton	= 2000 lb.
	= 907.1849 kg.
	= 0.9072 m. ton
1 metric ton	= 2204.6 lb.
	= 1000 kg.
	= 1.1023 short ton
1 picogram	= 10^{-12} gms
1 nanogram	= 10^{-9} gms
1 microgram	= 10^{-6} gms
1 milligram	= 10^{-3} gms
1 centigram	= 10^{-2} gms
1 gram	= 0.03527 oz.
	= 0.001 kg.
1 kilogram	= 35.27 oz.
	= 2.205 lb.
	= 1000 gm.

WEIGHT (continued)

1 lb./ac. = 1.12085 kg/ha
1 ton/acre = 2.24169 metric tons/ha
1 kg/ha = .89235 lbs/ac.
1 met. ton/ha = .44597 tons/ac. (892.8 lbs./acre)

PRESSURE

1 psi = .07031 kg/cm^2
= 7.031 kg/m^2
1 kg/cm^2 = 14.223 psi
= .9678 atmospheres
= 32.81 feet of water
= 28.96 inches mercury
1 atmosphere = 14.696 lbs/in^2
= 1.033 kg/cm^2
= 101.33 centibars
= 29.921 inches mercury (at 32 °F)
= 33.95 feet of water (at 62°F)
= 1033 cm of water (at 62°F)
= 76 cm of mercury (at 0°C)

TEMPERATURE °F = 9/5 (°C) + 32
°C = 5/9 (°F - 32)

°F	°C
0	-17.78
10	-12.22
20	- 6.67
30	- 1.11
32	0.00
45	7.22
50	10.00
60	15.56
65	18.33
70	21.11
75	23.89
80	26.67
85	29.44
90	32.22
95	35.00
100	37.78
105	40.56
110	43.33
115	46.11
120	48.89
212	100.00

°C	°F
0	32
5	41
10	50
15	59
20	68
25	77
30	86
35	95
40	104
45	113
50	122
100	212

SELECTED FIELD AND LABORATORY BIOLOGY METHODS

By

Milton Tunzi
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

U.S. ENVIRONMENTAL PROTECTION AGENCY

Region IX Laboratory

620 Central Avenue

Alameda, California 94501

SELECTED FIELD AND LABORATORY BIOLOGY METHODS

Table of Contents

Prepared by Milton G. Tunzi, Ph.D., EPA Laboratory
620 Central Avenue, Alameda, California 94501
(Comments and corrections would be appreciated)

First Edition October 1973
Second Edition February 1974

I. Sample Collection

Representative samples from any body of water are difficult to take. Directions can be given for a completely statistically valid approach (e.g., random sampling), but these would probably be beyond the resources of most laboratories. Furthermore, the approach should be determined in relation to the purposes of the study. This may preclude a random-sampling approach or make it unnecessary.

One of the best ways to assure that a sample is representative of a site (whether that site be chosen randomly as indicated above or arbitrarily as in this section) is to composite 3 or 4 or more equal-volume samples from each site. These can be put into a plastic bucket, mixed, and a container filled from this bucket.

A. Routine Sampling

1. Generally, the specific sampling sites are chosen because they are accessible, equally distant from each other, traditional sampling sites, or in locations of importance - near dischargers or in areas of water use. Samples may be taken above and below a discharge pipe, or they may be taken in the receiving water near the discharge pipe. Many times the location selected is one where a water quality standard may be exceeded.

2. Rivers, streams, estuaries

   Sites can be sampled from different depths, from different locations around the sides of a relatively-stationary large boat, and from different locations if a small boat is allowed to drift. Water moving past an

anchored boat can be sampled every half-minute, or longer time interval depending on time limitations at the station. A stream should also be sampled in this way from the bank, i.e., with samples taken from the stream throughout a given time period and composited. A wide-month, liter, plastic container attached to a pole can be used to reach further from shore so that flowing water can be more easily sampled, or so that the moving part of a stream can be reached. A wide stream which is not above boot-top in depth can be sampled by sub-samples from 4 or 5 locations in a transect across the stream. The subsamples must be taken upstream so that they will not be contaminated by the stream bottom stirred up from walking.

Compositing samples is suggested because then fewer samples would have to be analyzed. However, if variations within time or a small space are desired, then the samples could be kept discrete, i.e., not composited. Spatial or temporal variations at one one or more stations can then be used in statistical comparisons between the stations.

3. Lakes, reservoirs, and ponds

If five individual samples for nitrate analysis are to be taken from five-acre Lake X whose water is being mixed thoroughly (e.g., because of fall turnover), then one might choose locations so that all parts of the Lake would be represented. (Figure 1).

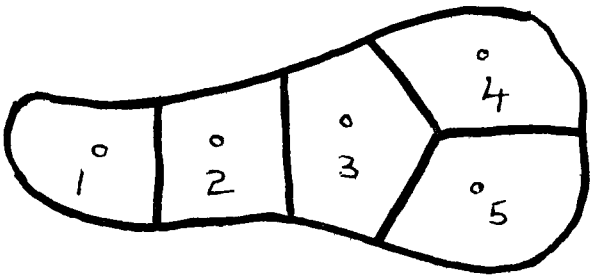


Figure 1. Lake X, Divided into Sections, Five Stations Shown.

In fact, one could take several samples in the area where he was to sample the single station and composite the several samples. For example, four or five subsamples could be taken in an area near sampling Station 1 (indicated on Figure 1 by circle), and the composited sample would represent Station 1. The compositing can be done from sample taken at different lake depths. This compositing approach is very useful where little time can be allotted to the execution of the sampling or where the project does not require a more sophisticated approach.

B. Random Sampling

Because of such variations as density, light penetration differences and the like, most waters would require a stratified random sampling approach.

1. Random Sampling, Spatial Approach

First the water body is arbitrarily divided into areas which are physically or geographically distinct. Then each area is divided into a grid pattern, and each section is numbered. The sections to be sampled in each area are selected by using a random numbers table. Only 2 or 3 locations are sampled in each physically or geographically distinct lake area. The same number of sections can be sampled in each area if the areas are approximately of the same size. The total number of samples would depend on resources and availability.

If a long channel is to be randomly sampled, it can be divided into separate sections each

with its own subsections. The difficulty with the subsection approach is that the areas are hard to delimit on the water, as there are no lines marked on the water surface.

2. Other random sampling approaches. As an alternative to a spatial design, temporal considerations also may be important. Time intervals also can be selected randomly with a new set of sections again selected by means of a random number table each time samples are taken.

   A given approach may be suitable for one type of measurement and not for another. Such factors as water movement, animal migration, and diurnal fluctuations cannot be overlooked in designing proper sampling.

C. Tests for Random Distribution

There are several ways in which a biological parameter can be tested to see if it is randomly distributed. The simplest way would be to compare the variance and means of samples taken from an area. Table 1 shows the significance of three such comparisons: $S^2 = \bar{X}$; $S^2 > \bar{X}$; $S^2 < \bar{X}$. These comparisons are valid if the distribution in only one location is being considered and if perhaps five or more samples have been taken in different sites of that specific location, and their mean and variance calculated (see Statistical Calculations).

The areal size, time span, and number of samples taken require further consideration. A biological parameter might be distributed randomly in one location and not in another, so the presumption that the results at one location apply to all locations in a water body is not valid.

The main advantages of a random distribution in a comparison of samples or in making statements about parameters is that confidence limits can be set which will delimit the true mean. However, if just a "yes or no" answer is required about whether there is a difference between samples, then non-parametrical statistical approaches can be used for both randomly and non-randomly distributed parameters.

D. Non-parametric Statistics

These procedures are very useful because no presumptions are made about sampling techniques, analytical methods, time of collection and, of course, distribution of the parameter. Furthermore, they are usually simpler tc calculate than the parametric tests. At the selected probability level, the results of the test give an answer to the question of whether or not there is a difference among the compared sampled means. Confidence limits containing the true population mean cannot be calculated using these tests. This is one of their main drawbacks. Suggested tests are below. The Kruskal-Wallis test is given in the Statistical Section. The others can be found in the references.

Test	
Mann-Whitney U - Test	An alternative to the t - test Sample numbers do not have to be equal
Kruskal-Wallis Test	Comparable to a one-way analysis of variance. Two or more samples can be compared. As above sample numbers do not have to be equal

Wilcoxon's Signed Rank Test	Use for detecting differences in paired samples
Spearman Rank - Correlation Coefficient (r_s)	This is the alternative to calculating the correlation coefficient for bivariate normal distributions (r)

References

Elliot, J. M., 1971. Some Methods for the Statistical Analysis of samples of Benthic Invertebrates. Scientific Publ. No. 25, Freshwater Biological Association. Ambleside, Westmorland, England. 144 pp.

Snedecor, G. W. and W. G. Cochran. 1962. Statistical Methods. Iowa State Univ. Press. Ames, Iowa. 534 pp.

Steel, R. G. D. and J. H. Torrie. Priciples and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.

Woolf, C. M. 1968. Principles of Biometry. D. Van Nostrand Co, Inc., Princeton, N. J. 359 pp.

Table 1 Significance of the variance and mean. Transform by converting each X measurement as indicated.

Conditions of the Samples	Distribution	Statistical Approach	Transformation
$S^2 = \bar{x}$ (1)	Random (Poisson	Use parametic statistics	If numbers are low transform each value $x = \sqrt{x}$ or $x = \sqrt{x + 1}$ if 0's are encountered
$S^2 < \bar{x}$	Uniform, Regular (Underdispersion or evenly spaced)	Use non-parametric statistics	None used
$S^2 > \bar{x}$	Contagious, Overdispersion (clumped or aggregated)	Use non-parametric statistics or transform so than $S^2 = \bar{x}$	Commonly encountered $x = \log_{10} x$ or $x = \log_{10} (X + 1)$, x is less than 1.0

1) $S^2 = \bar{x}$ means approximately equal

f upon transformation of the data, the variance is approximately equal to the mean then he data can be used in mormal statistical procedures such as t-tests, analysis of ariance, single and multiple regressions and correlations, analysis of covariance, etc.

^{2} is the variance
is the mean
is the standard deviation

II. Sample Collection Forms

On subsequent pages are sample collection forms, including directions for sample preservation. The sample preservation information is mostly from the EPA "Methods for Chemical Analysis of Water and Wastewater"; however, the determinations for which the same preservatives are used are placed contiguously.

The forms are suggestions only and can be modified according to needs.

SAMPLE COLLECTION FORM

INSTRUCTIONS

A. Project Director - Indicate measurements, location, date, sample points, sampler, time, samples to be taken and whether composite or grab. Indicate composite frequency. Check off these items on Sample Collection Form (one per sample).

B. Sampler - On same form fill in field data, correct date, and time of sampling if this information is different from that entered by Project Director (I).

x x x x x x x

Location _____ Date _____

Time _____

Sample Point _____

Sampler(s) _____

_____

_____

Field Measurements

___ Flow _____ ___ Clarity _____

___ DO _____ ___ pH _____

___ Settleable Solids _____ ___ Chlorophyll _____ (mls filtered)

___ Specific Conductance _____

_____

_____

C[1].	G[2].		C	G		C	G	
		BOD			Suspended Solids			Heavy Metals
		COD			Total Solids			Arsenic
		Coliform, Total			Volatile Solids			Chromium
		Nitrogen, Total			Sulfide			Copper
		NH_3-N			Total Organic			Cadmium
		NO_3-N			Carbon			Iron
		NO_2-N			Turbidity			Lead
		Odor			Pesticides			Mercury
		Oil and Grease			Oil Spill Sample			Nickel
		Phenol			Fish Bioassays			Zinc
		Phosphorus, Total			Algal Bioassays			Specific Conductance
		Cyanide			Benthic Sample			Others _____

C = Composite
G = Grab

Remarks: I. Project Director _____

II. Sampler _____

(See reverse side and attached pages for sample size, preservative and container type)

Check Equipment to be Taken for Sampling

(Always take - distilled water or deionized water, jugs, cubitainers, preservatives, rubber bulbs, Van Dorn Bottler or Kemmerer Sampler, pipettes, squeeze bottles, plastic bucket, rope, thermometer, towels, record book and/or Sample Collection Forms.)

_____ Flow - flow meter, weir apparatus

_____ DO - DO meter, buret, reagents, thiosulfate soln., starch, beaker, BOD bottles

_____ Specific conductance - meter, 2 cells (constants of 2x and 10x)

_____ Clarity - Secchi disk and line

_____ Benthic Samples - dredges, container, formalin

_____ Chlorophyll - GF/C filters, filter flask, vacuum pump (hand or electric), desiccant jars, styrofoam container, ice

_____ Algal count - container, formalin

Other Samples		Number of Containers
_____	Glass jugs	_____
_____	Cubitainers	_____
_____	Wide mouth plastic jars	_____

Preservatives, etc.

_____ Styrofoam container and ice
_____ Mailing container
_____ H_2SO_4
_____ 10 N NaOH
_____ HNO_3
_____ $CuSO_4 + H_3PO_4$
_____ $HgCl_2$ (Saturated solution)
_____ 2N Zn acetate
_____ 1:1 HNO_3

Container*	Parameter	Preservative	Holding Period	Volume Needed
NA	Dissolved Oxygen	Determine on site	NA	NA
Glass	Dissolved Oxygen (by titration)	2 ml $MnSO_4$ + 2 ml ALK-I	4-8 hours	300 ml
NA	pH, temperature, Settable solids, clarity	Determine on site	NA	NA
Cub. or Gl.	Metals, Total (one or all can be analyzed from same sample)	5 ml HNO_3 per liter	6 months	(For all parameters: 1 quart for individual analyses unless indicated differently. One gallon for combinations with same preservative)
	Metals, Dissolved (With arsenic HNO_3 interferes with reduction method; preserve arsenic samples with HCl)	Filtrate: 3 ml 1:1 HNO_3 per liter	6 months	
Gl.	Total organic carbon	2 ml H_2SO_4 per liter (pH 2)	7 days	
Gl.	Chemical Oxygen Demand	2 ml H_2SO_4 per leter	7 days	
Gl.	Oil and Grease	2 ml H_2SO_4 per liter-4°C	24 hours	1 gallon
	Petroleum Products	None required	Bring to Lab as soon as possible	1 quart

*Cub. = Cubitainer or polyethylene jar
Gl. = glass
NA = not applicable

Container	Parameter	Preservative	Maximum Holding Period	Volume Needed
Gl.	Pesticides, PCB	None required (Put teflon or aluminum foil under cap)		2 gallons
Gl.	Organo Phosphates	"	12 hours	2 gallons
Gl.	Chlorinated Hydrocarbons	"	2 days	2 gallons
	Phenolics	1.0 g $CuSO_4$/l + Conc. H_3PO_4 to pH 4.0 - 4°C (Use methyl orange indicator. At pH 4 it turns pink upon additions of H_3PO_4. Use 2 drops indicator/100 ml of sample or use pH meter)	24 hours	1 gallon
Cub. or Gl.	Cyanide	2 ml 10N NaOH/l	24 hours	1 gallon
"	Sulfide	2 ml 2N Zn acetate per liter	7 days	
"	Turbidity	None Available	7 days	
"	Solids	Refrigerate at 4°C	7 days	1 gallon
"	Acidity-Alkalinity, Color, Thres-hold Odor	Refrigerate at 4°C	24 hours	
"	Biochemical Oxygen Demand	Refrigerate at 4°C	6 hours	
"	Sulfate, Odor	Refrigerate at 4°C	7 days	
"	Fish Bioassays	Refrigerate at 4°C	6 hours	20 gallons of sample. 20 gallons of receiving water

Container	Parameter	Preservative	Maximum Holding Period	Volume Needed
Cub. or Gl.	Algal Bioassays	Refrigerate at 40°C	12 hours	
"	Chloride, Hardnedd, Specific Conductance, Fluoride, Calcium	None Required	7 days	
"	Algal Count	4% formalin	Indefinite	
Wide-mouth Jar	Benthic Sample	10% formalin	Indefinite	
	Kjeldahl Nitrogen	1 ml/l of saturated $HgCl_2$ sol-4°C	Unstable	
	Ammonia, Nitrate-Nitrite, Phosphorus	"	7 days	

III. Algal Bioassays

There are two general approaches in carrying out algal bioassays: (1) Using indigenous algae found naturally in a water sample (indigenous); or (2) adding a laboratory-grown single culture of algae. It is sufficient to say here that the use of indigenous algae in a bioassay is much easier than adding laboratory cultures. However, if the results of a bioassay are to be expressed as the dry weight of algae, this parameter can be more easily derived from single-specied bioassays. (There are many more advantages and disadvantages, to both approaches. These are discussed at length elsewhere [Tunzi, 1972]).

Directions to follow in carrying out algal bioassays will be divided into several sections:

Laboratory Bioassay directions
Cell Mass Measurement
Measuring Dry Weight
Measuring Algal Chlorophyll
Maintaining Algal Curtures in the Laboratory

A. Laboratory Bioassay Directions
(Bioassays utilizing Indigenous Algae)

Materials

Glass or polyethylene containers (e.g., cubitainers) for sample collection.
Ice chest, ice.
Filtration apparaters, vacuum pump.
Erlenmeyer flasks (250 or 500 ml each), acid rinsed (0.1$\overline{N}$H Cl), then rinsed with tap water and distilled water; water volume marks should be indicated on side of flask.
Waterproof labeling pens; black ink
Examples: Sanford's Sharpie #49; Scientific Products #P1226, Fine Tip Marker.

Foam rubber stoppers for erlenmeyer flasks; rubber stoppers for same.
Light box capable of 400 ft. candle illumination at 20°C; check uniformity of light with light meter.

Method

1. Collect samples in glass or polyethylene containers. Collapsible cubitainers are the most convenient. If the samples are from eutrophic water, about 1 quart is sufficient. Otherwise collect 1 gallon. If spiking of samples with nutrients or effluent is anticipated, then collect 1 gallon.

2. Keep the samples out of the sunlight. If necessary, surround samples by ice, but do not freeze. It is usually not necessary to ice if transport time is less than 1 hour.

3. If the samples are to be shipped a long distance, they can be put into styrofoam containers and surrounded by ice. The algae in the samples will remain cool and viable for about 12 hours during transit.

4. At the laboratory filter part of each sample for dry weight or chlorophyll determination (50 to about 400 ml is needed for eutrophic and 1 to 2 liters for oligotrophic waters).

5. Choose sample concentration. Suggested additions of effluent to receiving water are 1%, 5%, 10%, 50% of total volume. When preparing nutrients, prepare high concentrations so that additions will be 5 ml/liter of sample. Otherwise the distilled water used to dissolve the nutrients will dilute the sample so that comparisons with a control or with other nutrient additions is difficult (see Table 1, components of Macronutrient Medium for Algal Cultures, Section III-E.

6. Prepare about 1 liter of each concentration and mix well before adding the water to the replicates.

7. Prepare at least 4 replicates per sample type. Use black ink, waterproof pens for labeling.

8. Number the sample containing Erlenmeyer flasks with a waterproof marker pen. Replicates should be numbered; e.g., 1-1, 1-2, 1-3, 1-4; 2-1, etc. Number the flasks permanently on the frosted parts. If a flask has consistent erratic results compared to replicates of the same series, discard it.

9. If samples are to be incubated without additions, the flasks can be filled directly from the sample container. First shake sample well; then put 125 ml into the 250 ml flasks or 150 ml into 500-ml flask. Add the water to the volume marks on flasks. Extreme accuracy is not important.

10. Cover the flasks with foam rubber stoppers.

11. Take an initial cell-mass measurement on 2 of the 4 replicates (see Cell Mass Measurements).

12. Incubate the samples under 400 ft. candles of light at 20°C. If higher temperatures are used, the cultures grow too rapidly. There does not appear to be much advantage in intermittent lighting. The main point is uniform light. A light meter should be used to check that all areas of the incubation shelf are receiving approximately equal light (+ or -10%).

13. Measure the algal mass at about the same time every day.

14. Before measuring the mass, plug the flask with a rubber stopper and shake it vigorously. This promotes aeration and lessens the possibility of attached growth.

15. Expected growth curve, calculations, and reporting forms are shown in Figures 1 and 2. A completed reporting form is shown in the Statistical Section.

B. Cell Mass Measurement

Direct Cell Counting

Materials

Whipple micrometer reticule
Stage micrometer
At least 4 Sedgwick-Rafter Chambers
Pasteur pipette or automatic volume delivery pipette.

Procedure

1. Calibrate the microscope and Whipple disc (see Section 301 C, page 731, Standard Methods, 13th Edition), using a stage micrometer.

2. Fill each Chamber with water from one of the replicates by means of a Pasteur pipette or automatic volume pipette. Let the chambers settle for 5 minutes (Chamber volume is 1 ml).

3. Usually 2 strips are counted in each chamber and a factor is used to convert the number of cells counted to cells per ml for the sample. Make two counts of the cells in each chamber and record average.

4. Dilute aliquots from the flasks (with distilled water) if the cell concentration becomes too high. Serial dilutions may also be made to check accuracy of counting technique.

Turbidimetry by Turbidity Meter

Materials

Hach 2100 turbidimeter or equivalent.
Tubes for reading in Hach 2100.

Procedure

1. Calibrate the Hach Turbidimeter by means of the standard (the one supplied with the machine is adequate). The machine is set at the value indicated on the standard tube (usually 50 - 80 JTU's).

2. Read turbidity in each sample.

3. Obtain an average reading by watching the needle for 10-15 seconds. Fluctuations in readings are to be expected.

4. Use the same sample tube for each of the replicates of the same samples. It is not necessary to rinse the tube with distilled water between replicates of a single sample; however, the same tube must be well-rinsed or even washed between different samples.

5. When the maximum turbidity reading is reached (after incubation of sample), combine the water from the replicates, mix, and use for dry weight measurements (see Section on Weighing). (Maximum growth is reached when readings are approximately the same for 2-3 days [see Statistical Procedures for approach to evaluating differences in the samples].)

6. The lower limits of the detectable turbidity is about 2000 cells/ml, but 10 fold increases changes turbidity only about 2 units.

Absorbance by Spectronic 20

Materials

B & L Spectronic 20; Spec 20 tubes

Procedures

1. Set the wave length at 600 nm.

2. Using special Spec 20 tubes, read absorbance for each sample. Many tubes are required, since the sub-samples have to be poured back; generally 20-30 ml volume is utilized at each reading, and discarding this would deplete the incubating sample too drastically.

3. Take readings daily at about the same time.

4. When maximum value is reached and stabilized, express terminal values as dry weight. The water from the replicates can be combined to give enough volume to yield weight differences and the individual reading used for statistical comparisons (see sections on Weighing and on Statistical Treatment).

5. Depending on the size of the algal counts, the Spectronic 20 is good starting at about the 100,000/ml level. It is an instrument rather insensitive to any but large cell number changes.

In Vivo Fluorescence

Materials

1. Turner Model III Fluorometer (or equivalent) with an ultra-violet light source F4T5, the red-sensitive R-126 photomultiplier, Corning 5-60

primary filter and 2-64 emission filter. The general purpose photomultiplier can be used for dense cultures ($\approx 10^4$ cells/ml and up). The R-126 photomultiplier is sensitive down to 1000/ml. In contrast to the turbidity meter, 10 fold increases in cell number changes fluorescence 50-100 units.

Procedure

1. Zero the machine using the black plastic tube which comes with the machine. Check the zero calibration when changing from slit to slit or after reading every 3 or 4 samples. There are slits on the machine - 1 X, 3 X, 10X, 30X, the latter allowing the most light to pass through. Do not use the 1 X slit, as response of the machine is not linear with this slit. On top of the machine a dial reads from 0-100. Record both slit and dial values for each reading taken.

2. Establish a calibration factor for converting readings from one scale to another.

3. Follow sample incubation, etc. under 1-14 of the Laboratory Bioassay Directions, IIIA.

4. Shake the flasks thoroughly immediately prior to reading as clumping of algae can cause fluctuations in the readings.

5. Pour 5 ml of water directly into cuvette and take reading. Each reading only takes about 5 ml of sample, so that once the aliquot is read the water used can be thrown away.

6. Rinse tube as follows: for replicates of the same sample, rinse the tube with a subsample from the next replicate; between different samples, rinse the tube with distilled water.

7. Wipe the outside of the cuvette dry before inserting it in the holder.

8. Shake the next replicate, then take the reading of the tube in the machine. This should give about a 10-15 second period between readings. It is important that the time span be consistent.

9. When growth reaches a plateau (i.e., the amount of fluorescence does not seem to increase), combine the replicates for either chlorophyll a or weight measurement. Since algal chlorophyll fluorescence is the primary cause of sample fluorescence, chlorophyll a determination is the more reasonable one to make (see Measuring Algal Chlorophyll, beginning with Filtration, D-2). There is usually not enough sample for both measurements.

C. Measuring Dry Weight

1. Wash 4.25 cm GF/C Whatman filters by placing them in a pan of distilled water. Loose fibers will separate from the filters.

2. Place filters on a towel to partially dry.

3. Place them separately on a sheet of aluminum foil.

4. Dry them for three hours at 90°C. (Put into a desiccator if filters are to be stored for more than 5-10 minutes before weighing.)

5. Number them lightly on their edges with a soft lead pencil.

6. Weigh filters to nearest hundredth milligram. Handle the filters with tweezers, grasping the edges.

7. Put them into small envelopes with their weights and number written on the outside of the envelope.

8. When needed, filter as much sample as will go through the filter in about two minutes at low vacuum, less than 5 inches of mercury.

9. Record volume filtered in liters.

10. Double the filter, algal side inward.

11. Place filter on aluminum foil and dry for at least three hours at 90°C.

12. Remove them from oven and, using forceps to transfer, weigh them after they have cooled for about five minutes. Cooling in a desiccator may be desirable but appears to be of limited advantage.

13. Subtract original weight of dried filter from final weight and express results as mg/l of dry weight.

D. Measuring Algal Chlorophyll

Introduction

There are two practical approaches to measuring the concentration of indigenous algae in water. They can be counted directly or may be enumerated indirectly by determining the chlorophyll content of a sample of water (or performing some other mass measurement). The following method details procedures for measuring chlorophyll concentration.

Materials and Equipment for Laboratory Analysis

Whatman GF/C glass fiber filters, 4.25 cm diameter

Filter-holding apparatus: either
Millipore or Gelman
Covered small glass jars containing desiccant
Freezer
Scissors
Tissue homogenizer with teflon pestle: either
Kontes Glass Co. No. 885-380-0023; or
A. H. Thomas Co. No. 4288B
Acetone, (90% acetone, 10% water) spectrophotometric grade
Centrifuge tubes (if Kontes tissue homogenizer not used)
Centrifuge adapters (necessary only if Kontes tissue homogenizer used)
Pasteur pipettes
Beckman DU Spectrophotometer, or equivalent
Cuvettes (for Spectrophotometer), 1 cm or small volume 5 cm ones
Hydrochloric acid, 1N

Method

1. Sample Collection

It is best not to collect a single grab sample. Instead an integrated sample should be taken by collecting small (about 250 ml) equal-volume sub-samples at a given site and depth over a time period, such as 10 minutes. These sub-samples should be mixed together in a plastic busket and transferred to a transport container (e.g., a 1-gallon cubitainer).

Consult section on Sample Collection for further considerations on representative samples.

2. Filtration

1. As soon as possible after collection (the sooner the filtration the more valid the data; e.g., a sample stored in the dark on ice should be filtered

within 4-5 hours, if possible), filter under low vacuum as much water as will go through a Whatman GF/C filter within about two minutes.

2. If possible, prepare 4-5 filtrations of water from the same sample.

3. Record the water volume that has been put through each filter.

4. Double the filters, algal side inward and put them into a small jar with dessicant and then into the freezer for storage.

5. Extract for chlorophyll within three weeks of filtration.

3. Extraction Methods

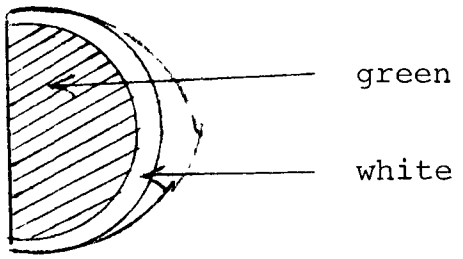


Figure 1. Folded Filter

1. Refer to Figure 1. Using scissors, carefully trim off the white border to the edge of the green algae-contining section. Discard white section. Cut each trimmed filter into smaller pieces, putting these directly into tissue grinder homogenizer.

2. Add 5 ml of acetone.

3. Grind filter with teflon pestle.

4. Ground-filter-and-acetone mixture should only get slightly warm to touch during the process. Move the tube slowly up and down while grinding, ceasing the grinding whenever the tube becomes warm to touch.

5. Keep ground-filter-and-acetone mixture out of strong light by covering it with a towel.

6. Pour the mixture into a centrifuge tube, cover with parafilm (or other cover), and shake well. If Kontes tissue grinder is used, the container may be centrifuged directly (if adapters are present), thus making it unnecessary to transfer the ground mixture to a centrifuge tube.

7. Let set in the dark for 20 minutes at least.

8. Shake the centrifuge tube well again after the 20-minute waiting period.

9. Centrifuge the tubes for 10 minutes at 2500 - 5000 RPM, preferably at the higher RPM's. Tap the tubes to bring particulate matter to the bottom. Recentrifuge.

10. By means of a Pasteur pipette, carefully draw off enough of the supernatant to fill a 1 cm cuvette (about 4 ml).

11. Take absorbance readings at 750 nm, blanking against 90% acetone. If above 0.005-0.008 Optical Density (O.D.), re-centrifuge.

12. Take absorbancy at 663 nm, blanking against 90% acetone.

13. Add 2 drops of 1N HCl to each cuvette and re-read absorbancy after 2 minutes at 663 nm. One cm cuvettes do not have to be shaken to disperse the acid, but it is necessary to shake those of larger dimension.

14. If the absorbancy of the sample is too high for the spectrophotometer scale, the sample can be diluted. Keep an accurate measure of the total amount of acetone used, as this volume is necessary for calculations.

4. Calculation of Results

1. The amount of chlorophyll a from algae can be calculated as follows:

$$\mu g \text{ chl } a \text{ per liter} = \frac{26.7\ (OD_b - OD_a) \times Ac}{W \times cm}$$

where: OD_a = optical density at 663 nm of extract after acidification (step 13 of Extraction), less the OD at 750 nm (step 11 of Extraction).
OD_b = optical density of extract before acidification (step 12), less OD at 750 nm (step 11).
Ac = volume of acetone in ml
cm = spectrophotometer cell path length in cm
W = volume of water in liters

2. Phaeophytin, a degradation product of chlorophyll, may be calculated as follows:

$$\mu g \text{ phaeophytin per liter} = \frac{26.7\ [1.7\ (OD_a) - OD_b] \times Ac}{W \times cm}$$

This value may be 0 or negative, indicating no phaeophytin in sample.

3. Total chlorophyll a per liter in any sample is the sum of the values obtained in 2 and 3.

4. If more than one filter was prepared, the chlorophyll concentration values from the 4 or 5 filters can be used to establish the standard deviation, standard error, and 95% confidence limits of the chlorophyll values for the sampling site (see Statistical Procedures).

Discussion

As was mentioned in the introduction, there are numerous ways to determine the amount of algae present in a sample. These include direct counting, weight measurement (biomass), trubidity determinations, and chlorophyll measurement.

Counting is a slow process. Its principal drawback however is that algae vary greatly in size, so that to get an estimate of the mass of algae in water each separate species has to be measured and the total volume obtained by multiplying the number of each species times its volume. (This value can be converted to mg/l of algae by assuming a specific gravity of about 1.0 for the algae.)

An extraction of algal chlorophyll is one of the standard methods of estimating standing crops in water (Strickland and Parsons, 1965). This is true because chlorophyll is a necessary constituent of green plants, serving as a catalyst in the initial carbon fixation process. One problem in determining concentration, though, is that the ratio of chlorophyll to cell mass can be changed, especially by varying the light intensity. The chlorophyll a to cell carbon ratios are in the range 1:40 to 1:100.

Biomass might also be determined. One disadvantage of this procedure is that the volume of material available for filtration is usually small so that the resulting weight of the cells retained by the filter is not too much greater than the weight of the filter itself. This causes a wide variation in results. If one wishes to determine cell weight, though, the method can be employed. Empirically it has been observed that the cell dry weight is approximately equal to two times the cell carbon (Maciolek, 1962).

A summary of the advantages of chlorophyll as a measure of mass would include the following points:

1. The amount of chlorophyll is determined spectrophotometrically. The precision of this determination is greater than that for any cell-count method.

2. Large volumes of water can be filtered to determine chlorophyll. Only one ml at most is used in direct counts (and hence is not too representative).

3. The green chlorophyll color of algae is the substance seen when one looks at algae in water; therefore, measuring chlorophyll in water usually is a direct way of quantifying the size of an algal bloom.

E. Maintaining Algal Cultures in the Laboratory

The EPA Report Algal Assay Procedure - Bottle Test (1971) available from Thomas Maloney, EPA, NERC, Corvallis, Oregon gives useful information on culturing algae. Pure cultures of algae can be obtained from NERC, Corvallis or from the Culture Collection of Algae, Dept, of Botany, Indiana Univ., Bloomington, Indiana. Direction are available in the Indiana University listing for media for specific algae.

General Directions

These are applicable for Selenestrum, Scenedesmus and mixtures.

1. Stock cultures can be kept viable for months if they are kept out of direct light. They may be stored at normal room temperature in a shelf of the laboratory where the light is constantly subdued or at least off at night. Cultures kept under constantly high light will go through a growth phase, exhaust nutrients, and usually die.

2. Use aseptic techniques for transferring uni-algal cultures. Pasteur pipettes, flasks and stoppers (or other covers) can be autoclaved or heated to 90°C if an autoclave is not available.

3. Table 1 shows a simple mixture of nutrients which will promote growth. Stock culture can be kept in polyethylene or glass bottles. Micro-nutrients are not needed as there appears to be ample present as contaminants in the chemicals. If they are desired, utilize those given in the EPA Corvallis publication (or add to 1 liter macronutrients 1 ml of a solution prepared by adding a small amount of bouillon cube to 100 ml water).

Table 1. Components of Macro-nutrient Medium for Algal Cultures

Component	Amount in g/l
$NaNO_3$	6 g/l
$CaCl_2$	0.6
$MgSO_4$	1.8
NaCl	0.6
KH_2PO_4	0.875
K_2HPO_4	0.375
$NaHCO_3$	10.0
$Fe(SO_4)_2(NH_4)_2 \cdot 12\ HOH$	860 mg Dilute in
Na_2 EDTA $\cdot$ 2 HOH	660 mg one liter

4. Add 10 ml of each chemical except the iron solution above to a two liter flask and bring volume up to 1 liter with ion-free or distilled water.

5. Cover the flask with a beaker and heat to 90°C or autoclave for 20 minutes.

6. Autoclave the iron solution or heat to 90°C. The iron solution should be kept in a screw-cap flask. Loosen caps when heating or autoclaving and tighten when cool.

7. Add 1 ml of iron solution to liter of the macro-nutrients when the latter has cooled.

8. The nutrient solution can then be dispensed to sterile smaller flasks (250-ml ones are suitable).

9. Inoculate the small flasks with the stock algae. Put under constant light of about 400 ft-candles. Solutions should be densely green in about five to seven days and ready for use.

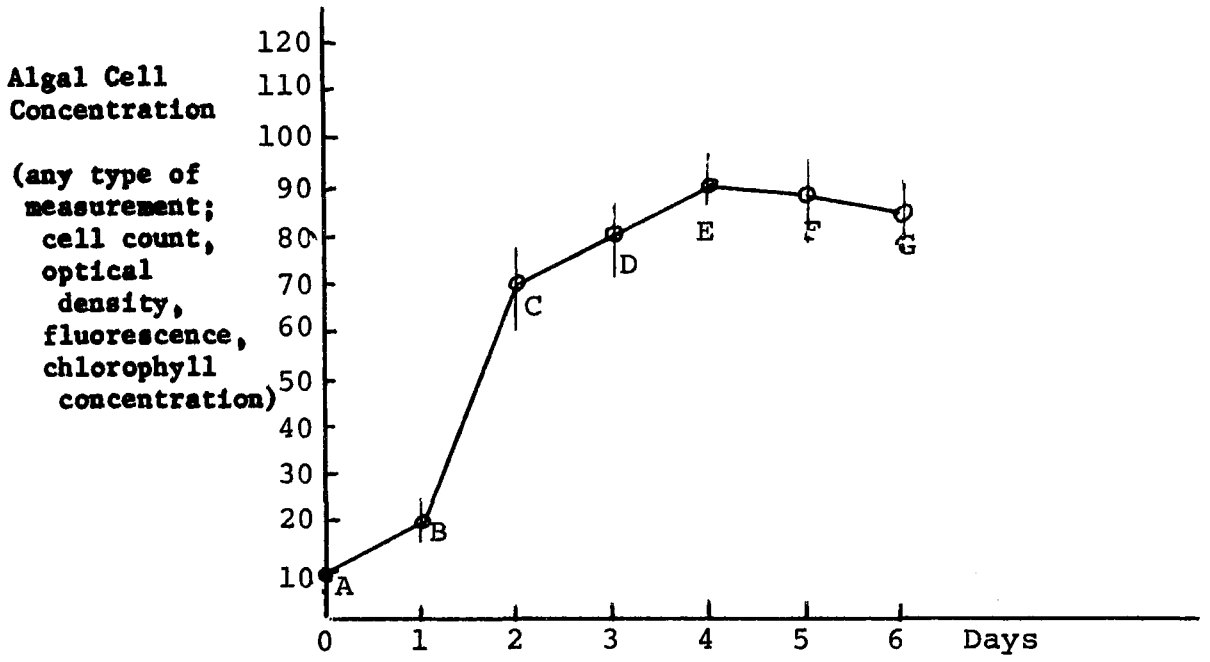
References

Anonymous, 1971. Algal Assay Procedure. Bottle Test, NERC Environmental Protection Agency. Corvallis. 82 pp.

Maciolek, J. A. , 1962. Limnological Organic Analyses by Quantitative Dichromate Oxidation. Res. Rept. 60. U.S. Fish and Wildlife Service. 61 pp.

Tunzi, M. G., 1972. Algal bioassays: Examples, advantages, and limitations of current approaches, 173-197 pp. in Proceedings of Seminar on Eutrophication and Biostimulation. California Dept. of Water Resources. Sacramento. 229 pp.

Strickland, J. D. H., and T. R. Parsons. 1965. A Manual of Sea Water Analysis. Fisheries Research Board of Canada. Bull, No. 125. Ottawa, Canada.



Algal Growth Data Sheet Parameters	Value from Figure
Initial chlorophyll Concentration	(A) 10
Peak chlorophyll Concentration	(E) 90
Increase in chlorophyll Concentration	(E minus A) 80
Days to reach peak	4 days
Maximum Growth rate $\hat{\mu}$, day $^{-1}$	$\hat{\mu} = \frac{\ln\left(\frac{x_1}{x_o}\right)}{t} = \frac{\ln\left(\frac{70}{20}\right)}{1} = 1.41$

x_o = cell concentration at beginning of maximum growth

x_1 = cell concentration at end of maximum growth

t = time

$\hat{\mu}$ = maximum specific growth rate, (day $^{-1}$)

Maximum growth rate is derived from the steepest part of the growth curve, utilizing the log of the cell concentration at the beginning and end of the curve.

Fig. 1. Typical algal growth response. The values are the means of the replicates, whose range are indicated by the vertical lines.

Figure 2 ALGAL GROWTH DATA SHEET

Sample		μg Chl a/l				
Location	Number	Average Initial Chlorophyll Concentration	Average Increase In Chlorophyll Concentration	Average Maximum Chlorophyll Concentration	Average Maximum Growth Rate $\hat{u}_b$, days^{-1}	No. of Days to Reach Peak

The results below connected by underlining are not different from each other at the 95% confidence level. Average based on the results of four replicates.

Sample Number Concentration Increase μg Chl a/l	
Sample Number Concentration Maximum μg Chl a/l	
Sample Number Maximum Growth Rate μ_b, day^{-1}	

Fig. 2. Data reporting sheet with multiple range section on the lower part. See statiscal section for elaboration.

IV. Statistical Procedures

A. Introduction

Statistics is a scientific method involving collection, analysis, and interpretation of numerical data. An understanding of basic statistical principles and procedures is helpful to both field and laboratory workers. The mathematics involved is simple except for advanced procedures which are infrequently used.

The data collected for statistical treatment are measurements or observations of a characteristic of a population. The population can be the cells in a series of flasks, the nitrate ions in a lake, the oligochaetes in the sediments of a bay, etc. Thus the population can have discrete physical boundaries or ones which the planner sets himself.

Almost without exception we cannot make all the desired measurements of a population, so that instead we take a sample from the population. From the sample mean, predictions can be made about the same characteristic in the entire population. By sample is meant a series of measurements, although in reality we would have to take a separate sample for each measurement.

Greek letters are used for population statistical terms and English letters for sample ones. The measurements from a population are called parameters and those of the sample called statistics.

Assuming that we have measurements from a population, the above can be clarified by the following table.

	Population Parameter	Sample Statistic
Mean	μ (Mu)	$\bar{x}$
Variance	σ^2	s^2
Standard Deviation	σ (Sigma)	s

B. Definitions

1. Mean ($\bar{x}$). The average value calculated by dividing the sum of the measurements by the number (n) of measurements.

2. Variance (s^2). The variability or spread of the data about the mean (See Figure 1).

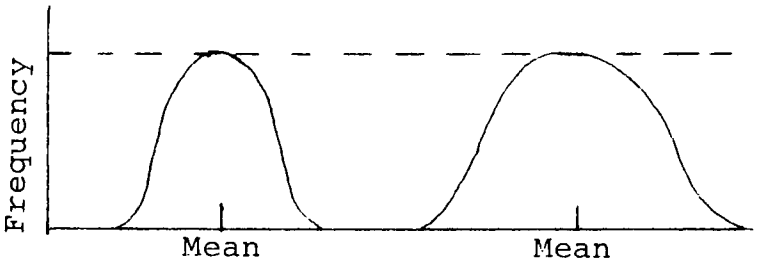


Figure 1. Two sample measurements with equal means but differing variances.

3. The standard deviation (s). The square root of the variance.

4. The standard error or the standard error of the mean ($S_{\bar{x}}$). The standard deviation of the sampling distribution of means.

5. Degrees of freedom - usually equal to N -1.

C. Calculations

The calculations for the above statistics are very simple.

Given the data below collected from a population with individual measurements listed under x.

x	$x - \bar{x}$	$(x - \bar{x})^2$	x^2
11	-2	4	121
12	-1	1	144
15	2	4	225
16	3	9	256
11	-2	4	121
65	0	22	867

$(x-\bar{x})^2$ is the sum of the squared deviations which is called the sum of the squares (SS).

x^2 is calculated because it is used in the working formula for the variance.

The mean being: $\sum_1^n x$

$$\bar{x} = \frac{\sum_1^n x}{n} = \frac{65}{5} = 13$$

The variance is:

$$s^2 = \frac{\sum_1^n (x-\bar{x})^2}{n-1} = \frac{22}{4} = 5.5$$

The working formula is simpler because the sum of the squares does not have to be calculated.

$$s^2 = \frac{\sum_1^n x^2 - \frac{\left(\sum_1^n x_1\right)^2}{n}}{n-1} = \frac{867 - \frac{(65)^2}{5}}{4} = 5.5$$

Standard deviation

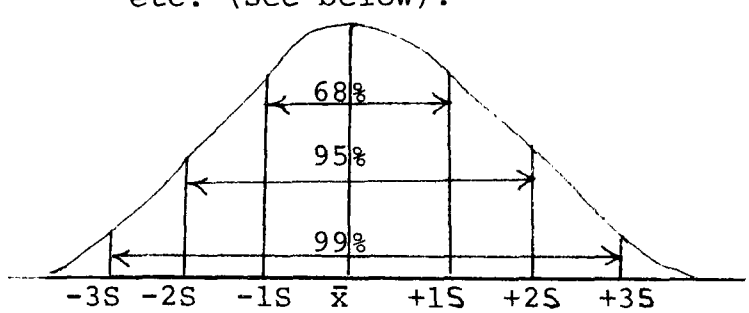
$$s = \sqrt{s^2} = \sqrt{5.5} = 2.35$$

Standard error

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} = \frac{2.35}{2.24} = 1.05$$

By use of the standard deviation confidence limits for the measurements can be set:

$\bar{x} \pm 1S$ includes 68% of the sample measurement etc. (see below).



This assumes that the sample measurements are normally distributed.

Confidence limits of the population are much more important. That is we want to set limits which bracket the true population mean or average (μ).

This can be done by using the standard error $S_{\bar{x}}$ and t table values for the degrees of freedom in our sample.

$\bar{x} \pm s_{\bar{x}}$ will include the true population mean (μ) 68 out of 100 times.

$\bar{x} \pm (t_{0.05}) s_x$ will include μ 95 out of 100 times.

$\bar{x} \pm (t_{0.01}) s_x$ will include μ 99 out of 100 times.

The difference between sample confidence limits and population confidence limits must be clearly understood.

Using the last formula in the above data:

$\bar{x} \pm (4.60)(1.05) = 13 \pm 4.83$

The $t_{(0.05)}$ and $t_{(0.01)}$ are found in a t table for a range of n-1 values (a few values are given below).

	Degrees of Freedom (n-1)				
	6 DF	5 DF	4 DF	2 DF	1 DF
$t_{(0.05)}$	2.45	2.57	2.78	4.30	12.71
$t_{(0.01)}$	3.71	4.03	4.60	9.93	63.66

By utilizing the t values for various degrees of freedom, we can see the importance of high numbers of replicates in sampling.

D. Group Comparison of Two populations

1. A comparison consist of two steps.

   a. A t test to determine if the sample means come from one or two populations.

   b. Confidence limits can be set for the sample means if the t test is significant.

2. Special formulas for group comparisons

a. $\mu = \bar{x} \pm t\,(0.05)\,\frac{s_p^2}{n}$

Note: If the t test is not significant then both samples come from the same population.

The confidence limits for the population means may overlap slightly even when the t test is significant.

b. Pooled variance

$$s_p^2 = \frac{\sum_1^n x^2 - \frac{\left(\sum_1^n x_1\right)^2}{n_1} + \sum_1^n x^2 - \frac{\left(\sum_1^n x_2\right)^2}{n_2}}{(n_1-1) + (n_2-1)}$$

These formulas can be used whether or not $n_1 = n_2$.

3. Example of a group test

x_1	x_2
32	17
31	35
52	22
44	24
x 159	98
x^2 6625	2574
$\bar{x}$ 39.75	24.50

$$s_p^2 = \frac{6625 - \frac{(159)^2}{4} + 2574 - \frac{(98)^2}{4}}{(4-1) + (4-1)}$$

$$s_p^2 = \frac{6625 - 6320 + 2574 - 2401}{6}$$

$$s_p^2 = \frac{478}{6} = 79.7$$

$$t = \frac{39.75 - 24.50}{79.7(1/4+1/4)} = \frac{15.25}{39.85} = 2.42$$

For 6 degrees of freedom $t[0.05] = 2.45$. Therefore, there is no difference between the set of data.

E. Comparison of Two Groups by Pairing

1. If two samples are not independent, then a pairing test can be used to compare them. Of course, $n_1 = n_2$. Generally, high values in one sample are associated with high values in another.

2. Example of the data from a pairing test:

x_1	x_2	$x_1 - x_2$ difference	d^2
10	19	-9	81
9	18	-9	81
8	17	-9	81
11	19	-8	64
14	22	-8	64
12	18	-6	36
$\sum$ 64	113	-49	407
$\bar{x}$ 10.64	18.83	$\bar{x}_d$ = -8.17	

$$\left(\sum_{1}^{n} d\right)^2 = 2401 \qquad \sum_{1}^{n} d^2 = 407$$

$$\text{Variance of the difference } s_d^2 = \frac{\sum_{1}^{n} d^2 - \frac{\left(\sum_{1}^{n} d\right)^2}{n}}{n - 1}$$

$$s_d^2 = \frac{407 - \frac{(49)^2}{6}}{5} = \frac{407 - \frac{2401}{6}}{5} = 1.4$$

The standard error is:

$$s_{\bar{x}d} = \sqrt{\frac{1.4}{6}} = \sqrt{0.233} = 0.48$$

$$t = \frac{|\bar{x}_d|}{s_{\bar{x}_d}} = \frac{8.17}{0.48} = 17.0$$

Since the t value of 17.0 is greater than the t value for 5 degrees of freedom (0.05), there is a significant difference between the sample means.

The 95% confidence limits for the difference between the samples can be calculated by using the following formula:

$$\mu_d = \bar{x}_d \pm t(0.05)\sqrt{\frac{s_d^2}{n}}$$

F. Comparison of data from more than two groups

1. Analysis of variance is the technique used to compare one characteristic from three or more populations.

   Three or more populations cannot validly be compared by the sequential use of a t test. It is especially bad to single two groups out of a large number and subject them to a t test to see if they differ.

   The completely randomized design is used for comparing the <u>means</u> from three or more populations.

2. The following calculations for unequal sample size can also be used when the same number of measurements are made for all samples:

Sample

	1	2	3	
	10	6	10	
	11	12	12	
	12	14	10	
	13		9	
$\sum$ $\bar{x}$	46	32	55	$\sum$ = 133
$\sum$ x^2	534	376	621	$\sum$ = 1531
n	4	3	5	$\sum$ = 12
$\bar{x}$	11.50	10.67	11.00	
$\frac{(\sum x)^2}{n}$	529	341.3	605	$\sum$ = 1475.3

Correction Factor = $\frac{(133)^2}{12}$ = 1474.1

Total Sum of Squares = 1531 - 1474.1 = 56.9

Treatment Sum of Squares = 1475.3 - 1474.1 = 1.2

Analysis of Variance Table

Source of Variation	DF	SS	Mean Square	F
Total	11	56.9		
Treatment	2	1.2	0.6	0.097
Error	9	55.7	6.2	

$$F = \frac{\text{Treatment mean square}}{\text{Error mean square}}$$

F values for 2 degrees of freedom in the numerator and 9 in the denominator for the 0.05 probability level is 4.3, much higher than our F value. Therefore, there is no significant difference between our sample means.

3. If the F value is significant, a multiple range test must be used to determine which samples actually differ. Duncan's new multiple range test for equal replication (p. 107) and unequal replication (p 114) are good ones to use (Steel and Torrie, 1960).

G. Non-parametric methods

These approaches are useful when it is not certain that the normality of the population distribution and its means and variance are the same as that of the sample.

One of the more useful tests is the Kruskal-Wallis test which compares medians from 2 or more populations. An example of this ranking test is given below:

Data Set I	Rank	Data Set II	Rank
6	2	21	9
8	3.5	15	8
8	3.5	4	1
12	6	11	5
14	7		
30	11		
23	10		
Median = 12	n_1 =7 R_1 =43	Median = 13	n_2 =4 R_2 =23

Median-the middle value for odd number of values, and the mean of the two middle values for even number of values

$$\frac{11 + 15}{2} = \frac{26}{2} = 13$$

An H value is then calculated where:

k = number of samples

T = total number of measurements in all sets

$k-_1$ = degrees of freedom

R = sum of the rank

n = number of measurements

12 = a constant

$$H = \frac{12}{T\ (T+1)} \sum_{1}^{k} \frac{R_i^2}{n_i} - 3\ (T+1)$$

For the above data:

$$H = \frac{12}{11\ (12)} \frac{43^2}{7} + \frac{23^2}{4} -3\ (12)$$

H = 0.035

The hypothesis made is that the populations are identical. When the H value is greater that the chi-square value for k-1 degrees at the 0.05 probability level, then there is a difference between the sample medians. For 1 degree of freedom this = 3.84. So the hypothesis is accepted. (Chi-square tables are found in most mathematical handbooks and statistic books.)

TABLE 1 - BIOASSAY DATA WITH MULTIPLE RANGE STATISTICAL PRESENTATION

ALGAL GROWTH DATA SHEET

Sample 3/17/70 µg Chl a/l

Location	Number	Average Initial Chlorophyll Concentration	Average Increase In Chlorophyll Concentration	Average Maximum In Chlorophyll Concentration	Average Maximum Growth Rate $\hat{\mu}_b$, days $^{-1}$	No. of Days to Reach Peak
Redwood City Sewage Treatment Plant	12	7.2	2.0	9.2	0.13	6
	1	2.0	33.6	35.6	0.94	6
	2	2.0	0.0	2.0	0.00	0
downstream location	3	2.1	66.6	68.7	1.09	3
	4	2.1	69.8	71.9	0.61	3
	5	2.5	40.3	42.8	1.67	3
	6	2.8	26.1	28.9	1.57	3
San Francisco Bay	15	3.0	6.8	9.8	0.93	2

The results below connected by underlining are not different from each other at the 95 percent confidence level. Average based on the results of four replicates.

Sample Number	2	12	15	6	1	5	3	4
Concentration Increase µg Chl a/l	0.0	2.0	6.8	26.1	33.6	40.3	66.6	69.8
Sample Number	2	12	15	6	1	5	3	4
Concentration Maximum µg Chl a/l	2.0	9.2	9.8	28.9	35.6	42.8	68.7	71.9
Sample Number	2	12	4	15	1	3	6	5
Maximum Growth Rate $\hat{\mu}_b$, day^{-1}	0.00	0.13	0.16	0.93	0.94	1.09	1.57	1.67

V. Fish Bioassays

Sources of Fish

1. Collection

Test fish may be collected from a large body of water using a seine; from a small slough, one can use dip nets. Usually a collection permit is required (get this from state fish and game departments).

Since collection of suitable and adequate number of fish is somewhat uncertain, it should be done only if time is of little importance, if the locations of the desired fish are well-known, and if there is no other way to obtain fish.

After collection, place fish in a suitable transport container. A five-gallon plastic bucket with a snap-on lid can hold 100 small (up to 2 inches) fish for short distances.

If one collects his own fish, he must have several good battery-operated aerators (e.g., the Jorgensen portable aerator - $7.00; Lewis Air Pump - $3.50). Take extra batteries and check at least every hour to see that they are not run down. Aerate fish on the way back to the laboratory.

Aeration is accomplished by connecting aerator to a flexible line with an airstone at the end. The airstone should be weighted or it will float to the water surface. A large (No. 10) rubber stopper with a hole in it (to put the tube through) will hold the aerator under water.

2. Purchase

Commercial aquarium and fish stores generally charge too much to make them a reasonable source of fish. Names of dealers who supply fish for bioassay are generally available from agencies such as State Water Resources Control Boards and fish and game agencies or from other persons who carry out fish

bioassays. The price per fish delivered is usually 20-50 cents, depending upon the species. This is normally the most economical way to get fish. Be sure not to acquire more fish than needed. It is usually easier to purchase fish in lots as required than it is to maintain fish for many weeks in an expectation that they might be needed.

Possible sources of fish from agencies include: (Normally the hatcheries supply fish only to other public agencies)

Striped Bass
Bureau of Reclamation, Tracy
Phone 209-935-3122
Rainbow Trout
American River Hatchery
Phone 916-351-0314
Salmon, Steelhead Trout
Nimbus Fish Hatchery
Phone 916-351-0383
Black Bass, Blue Gill, Shad, Catfish
Elk Grove Hatchery Phone 916-685-9555

Two commercial fish dealers in the San Francisco Bay area are:

William Putman
5449 Modoc St. Richmond, CA 94804
Alex Fish Company
2235 Juniperberry Drive
San Rafael, CA

A list of commercial fish dealers in California is available from the California Fish and Game Department.

Recommended Species

Ideally, the best fish to use are the most abundant or economically significant young small ones found in the receiving water area. However, this may be impractical or impossible to carry out as they would be too difficult to catch or only available during specific seasons.

A standard test species available throughout the year would make comparisons between tests more meaningful.* Fish most commonly used in California are:

	euryhaline
3-spine stickleback	Gasterosteus aculeatus
Threadfish shad	Dorosoma petenese
Killifish	Fundulus parvipinnis
Striped bass	Roccus saxatilis

	Fresh Water
Golden shiner	Notemigonus chrysoleucas
Channel catfish	Ictalurus punctatus

Maintenance of Fish

1. Disinfection

   a. A new group of fish should be disinfected by putting them (for about 5-10 minutes) in water containing both .025 ml/l of formalin and 0.05 mg/l malachite green (Leteux and Meyer, 1972).

   b. Fifty fish can be put into approximately two gallons of water.

   c. Watch them carefully, and remove them immediately if they show signs of distress (floating up slightly sideways).

2. Aeration

   a. Before adding fish to water, aerate water for 12-24 hours.

*Table 3 lists animals suitable for bioassay in Hawaii

b. A twenty-gallon aquarium can hold 100 small fish if it has two activated charcoal filters and one to two airstones running constantly. Sometimes it is better to replace the water or part of it every three or four days, but aerate the water for 12-24 hours before adding it to the tank.

c. One example of an activated charcoal filter is the large-size Halvin which attaches on the side of the aquarium. Examples of electric air pumps are the Silent Giant ($15), Oscar and Star ($8). Activated charcoal should be changed every two days. The charcoal can be reused if fired in an oven at 450°C for an hour. Less heat will not destroy the organics absorbed in the charcoal surfaces.

d. If one uses a compressor as an air source, the air should first be passed through one tube: the first half holding non-absorbant cotton and the second half holding activated charcoal. The cotton and charcoal should be changed every month.

e. A large sand filter fiberglass system is shown in Figure 1. This can be used for 200-300 fish. The pump can be run constantly. Its size should be sufficient to circulate the water in the tank once per hour.

   Back-flush the sand filter every 3 weeks. Turn off the pump when feeding the fish.

3. Temperature for Fish Maintenance

   a. Cold-water fish should be kept at 13-14°C in order to remain disease-free. Warm-water fish also are usually less apt to contact disease when kept at these cool temperatures.

b. There are several ways to maintain these cool temperatures. One of these is a water bath with a refrigerant system. Another is a walk-in box with a refrigerant system.

4. Feeding Fish During Maintenance Period

a. When feeding fish, turn off the aerators and any filtration system (including activated charcoal).

b. Throw in food slowly until fish cease eating; this usually takes 10 to 15 minutes.

c. Look at the individual fish and remove any that have any discoloration and, of course, any dead ones. Generally only 1 or 2 fish will die out of a hundred, and these in the first days after delivery.

d. Fish should be fed 3 times a week; however, they can do without feeding on the weekends.

e. Do not over feed.

f. Fish food may be purchased as pellets or in frozen form. Fish food is available in bulk in pellet form of various sizes. No. 2 is suitable for small fish, but larger pellets can be ground in a mortar if only one size is available. Brine shrimp can be purchased frozen. Chunks can be broken off as needed. Put the frozen chunks into a beaker of water until they melt apart. Stir them and let the shrimp settle to the bottom. Pour off the supernatant water, add more water and repeat the process. In this way, less debris is added along with the shrimp. (If one is feeding fish in large tanks, the brine shrimp chunks can be thrown in directly).

f. Fish are not to be fed 2 days before the commencement of any test.

5. Holding and Dilution Water

Most fish are either marine or fresh-water, but some fish can live in water of varying salinity. These are termed euryhaline fish. If freshwater discharges into a freshwater receiving water are being tested for their toxicity, then a freshwater species can be used and a marine species for saline discharges into the ocean.

However, when low-salinity water is discharged into an estuary or the ocean, then a euryhaline species is the appropriate one to use. The euryhaline species can be kept in a 1:1 mixture of marine and tap water. It will withstand without great stress transferral from this mixture into both the effluent and sea water. These extremes in salinity would be present in the test waters because the concentrations used would include both sea water and effluent and mixtures of the two.

Fresh water holding water and the dilution water can be tap water that has been aged or aerated for 12 hours. For some of the reasons given above, there are usually two controls, one the holding water and the other the dilution water. If the fish are kept in the holding water within the laboratory-maintained temperature range, then the fish left in the holding water can be considered controls.

When both the receiving water and the effluent from the discharger are suspected to be toxic, a double control can be made. Dilution water could be river water upstream of the effluent in which a double control should be used. Control 1 being the river dilution water; control 2 the aged tap water. Sometimes results will vary if you use existing receiving water as a diluent instead of

using tap water as diluent. For example, when salts are high in receiving waters, this may have a positive or negative effect on effluent toxicity. If one has two controls (river water and tap water) and there is mortality due to the receiving water (river) rather than the effluent, use of the second control, tap water, will make this obvious.

Bioassay Procedure

Materials Required

Bioassay containers. These may be five-gallon (19-liter) aquaria, pickle jars or battery jars which are available in sizes up to one gallon; the size depends upon size of fish - one gm fish per one liter water; fish normally require 10-15 liters per test sample.
Container-cleaning facilities (large thick rug; garden hose).
Aluminum foil or lids for bioassay containers.
Temperature controllers. (Capable of maintaining 20°C $\pm$ 2°C for warm-water fish and 15°C $\pm$ 2°C coldwater ones).
Aeration device
Dissolved oxygen meter. DO can be measured by siphoning but then large-volumed containers are required. See Fig. 2.
Thermometer. (Either a recording thermometer or a small thermometer in a jar of water).
Optional: devices for measuring pH, conductivity, turbidity, and hardness.
Data recording sheets (See attachment, Figure 3)
Bioassay organisms (e.g. fish) [fish must be held at experimental temperatures for 10 days prior to commencement of bioassay for legal purposes].

Method

1. Scrub bioassay glass containers clean and rinse them well with tap water. If the containers are large, it is safer to do this on a large thick rug, using a light garden hose for rinsing the jugs. This is best done out of doors on a cement platform.

2. Let the containers drain for about one hour, then let them air dry inside the laboratory. After they are dry, cover them with aluminum foil or lids to keep dust-free, or store upside down.

3. Normally ten fish are added to each container. The weight of the fish cannot exceed 1 gram per liter of water. If fish are too large for 1 container, put five fish into each of two separate containers containing the same sample solution.

4. There are several ways to increase the reliability of the tests:

   a. Increase the number of fish from 10 to 20 per container (remaining consistent with the weight to volume restriction above).

   b. Prepare replicates of each concentration so that there would be two or more of each test solution (with 10 fish per container).

   c. Prepare concentrations with closer increments of toxicants e.g., instead of 10%, 20%, 30% there would be 10%, 15%, 20% etc. additions.

5. a. Preparation of concentrations

   The graph shown in Figure 4 is a standard plot of log of concentration versus regular arithmetic increments. This plot is based upon experimental results which show that effect of a toxicant upon an organism is logarithmic rather than arithmetic. That is to say that, in general, if one doubles the concentration one does not double the mortality.

   Actual additions of toxicant are in logarithmic increments. An excerpt from Standard Methods is given in 5b. It includes Table 1 which shows some log increments; a more complete

range is expressed in Figure 4. Figure 4 shows concentrations ranging from 100% to 10%. If a wider range of concentrations were to be employed, then several-cycle semilog paper would be used - e.g., a range of 100% to 0.1% would require four-cycle semilog paper - or divide values in Fig. 4 by 10 or multiples of 10.

If possible, the actual concentrations chosen would be based on a preliminary test of 12-24 hours with toxicants added in concentrations covering a wide range of values. For an unknown substance this might be 100%, 50%, 10%, 1%, and 0.1%. Regardless of the preliminary results, if possible, always include a 100% full strength test sample because many toxicity standards are based on percent survival in the pure test sample.

With experience and a preliminary test, the concentrations can be selected so that containers very close to the TL_{50} value will be the most numerous. A preliminary test using 2-4 fish per concentration can be carried out if the test material does not degrade. For example, if the preliminary test using two fish per liter showed the following results

Concentration of Test Solution	Survival
100%	0
50	0
25	1
10	2
1	2

Then the following concentrations could be set up:

100% (Optional)
56
32
24
18
10

b. Excerpt from Standard Methods, 13th Ed., p. 565 "Although a TL_{50} may be determined by testing any appropriate series of concentrations of the substance or waste assayed, the geometric series of concentration values given in Table 1 is often most convenient and has been widely used. These values can represent concentrations expressed as percent by volume or as milligrams per liter, etc.; they may all be multiplied or divided, as necessary, by any power

TABLE 1: GUIDE TO SELECTION OF EXPERIMENTAL CONCENTRATIONS, BASED ON PROGRESSIVE BISECTION OF INTERVALS ON LOGARITHMIC SCALE

Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
10.0				
				8.7
			7.5	
				6.5
		5.6		
				4.9
			4.2	
				3.7
	3.2			
				2.8
			2.4	
				2.1
		1.8		
				1.55
			1.35	
				1.15
1.0				

of 10. For example, the two values in the first column may be 10.0 and 1.0 as shown, or they may be 100 and 10, or 1.0 and 0.1, with the values in the other columns changed accordingly. The values of

the series 10.0, 5.6, 3.2, 1.8, and 1.0 (i.e., Cols. 1-3), or 10.0, 7.5, 5.6, 4.2, 3.2, etc. (Cols. 1 through 4), are evenly spaced when plotted on a logarithmic scale."

6. At the beginning of the bioassay, measure dissolved oxygen (DO) in each container. If it is below 4 mg/l, aerate that container until the DO is above 4 mg/l.

7. Additional optional measurements (in order of importance) include pH, conductivity, turbidity and hardness (titration, expressed as EDTA as $CaCO_3$). Figure 3 shows a blank data sheet. Figure 5 shows a typical data sheet with observations recorded.

8. Record the temperature daily (on Data Sheet, Figure 5, range of temperature is recorded following reading on 7-day recording thermometer).

9. Keep room semidark and do not let people wander needlessly in to frighten fish.

10. When transferring fish, do so gently so as not to harm them.

11. Add fish in groups of two to the jugs. Random placement of jugs and random addition of fish is recommended (see section on Random Sampling).

12. Using data sheet, record mortality and D.O. at least every 24 hours along with any other information about the bioassay that may be subsequently of interest. Remove dead fish as soon as they are observed.

Calculation of Results

1. The TL_{50} (concentration of toxicant killing 50% of the fish) at 96 hours should be calculated by plotting toxicant concentration on the ordinate scale of semilog graph paper and survival on the abscissa (normal scale axis).

For example, if the 96-hour results were obtained from a toxicity test as below (in Table 2) the TL_{50} can be seen from Inset in Figure 5 to be 68%.

Table 2. Survival of Fish vs. Toxicant, Typical Data

Survival (per 10 fish total)	% Toxicant
0	100
3	75
6	65
9	56
10	42
10	24
10	10

Statistical Treatment of Fish Bioassay Results

The TL_{50} value can also be calculated by using the Reed-Muench Method (Woolf, 1968). This method also allows one to calculate the 95% confidence limits which contain the true TL_{50} value.

Utilizing the data given on the sample record sheet for the "Northwest STP", the calculations are given in Figure 6. Natural logs can be used in place of logs to the base 10, if this is more convenient. If the lowest dose in mg/l or percent volume of toxicant is less than one, multiply the dose values by 10 or 100 as logs values less than one are negative. Then divide the resulting final values by the same multiple. Express the TL values as whole numbers in the example given.

REFERENCES

Leteux, F. and F. P. Meyer. The Progressive Fish Culturist 34. 1972. "Mixtures of Malachite Green and Formalin for Controlling Ichthyophthirius and other Protozoan Parasites of Fish."

Woolf, C. M. 1968. Principles of Biometry. D. Van Nostrand Company. Princeton, N. J. 359 pp.

Table 3
TEST ORGANISM SUITABLE FOR THE STATE OF HAWAII

University of Hawaii at Manoa

Department of Zoology
Edmondson Hall • 2538 The Mall
Honolulu, Hawaii 96822

Suggested Native Hawaiian Fauna
for Aquatic Bioassay

(John A. Maciolek - Associate Professor)

The following fresh and brackish waters animals are available on most islands in Hawaii and generally can be kept without undue difficulty in aquaria and holding tanks.

A. Freshwater species.

1. Shrimp: Atya bisulcata = opae kalaole, "mountain opae". Occurs in fast-flowing streams to about 3,000' elevation. Very abundant in pristine streams, but on Oahu, it is common only at higher elevations. Filter-feeds on stream seston and epilithic algae. Normally completes its life cycle in freshwater but larvae can tolerate salinity. Size: to about 2".

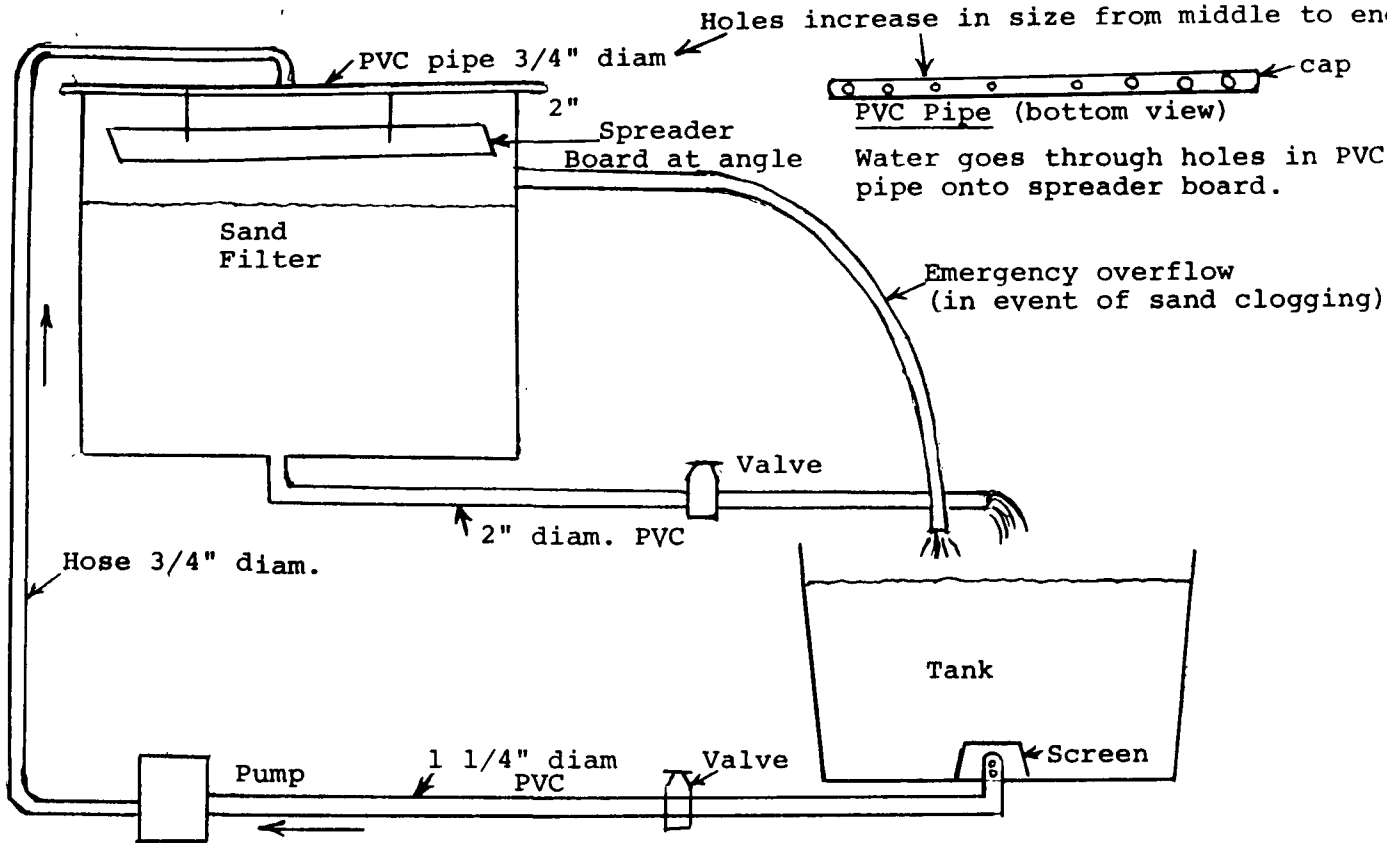
2. Fish: Awaous stamineus = o'opu nakea and Sicydium stimpsoni = o'opu nopili. Both species are abundent in the lower to middle reaches of perennial streams on neighbor islands; much less common on Oahu. Larvae develop in ocean and migrate upstream as post-larvae (hinana), often in great numbers, during several months of the year. Juveniles and adults do not tolerate saline water. Feed on benthic algae (especially nopili) and small invertebrates. Size: hinana about 1"; nakea adult to 12"; nopili adult to 7".

B. Brackish water species: the following shrimp and fishes are broadly euryhaline (freshwater to seawater).

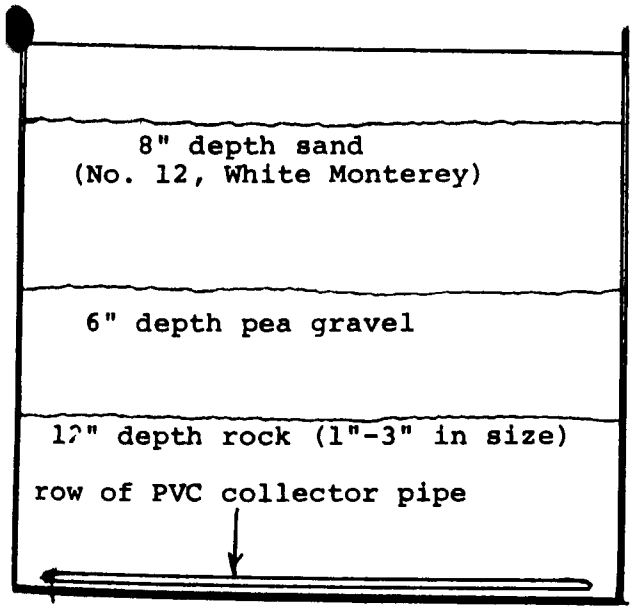
1. Shrimp: Palaemon debilis = opae huna, "glass shrimp". Most common in estuaries and brackish shoreline ponds, but is also found in most protected inshore marine areas. Omnivorous, feeds on plant materials, detritus, etc. Can complete its life cycle in brackish water. Size: to 1.5".

2. Fish: Kuhlia sandvicensis = ahole, aholehole. Occur in estuaries and inshore marine areas. Juveniles (to 3") invade lower reaches of streams. Carnivorous; predaceous on invertebrates (shrimps, worms) and small fishes. Size: to 12".

3. Fish: Mugil cephalus = amaama, grey mullet. Habitat similar to Kuhlia, but is herbivorous--feeding on phytoplankton, bottom sediments, etc. Fry and small juveniles common in estuaries. Size: to at least 2 feet.

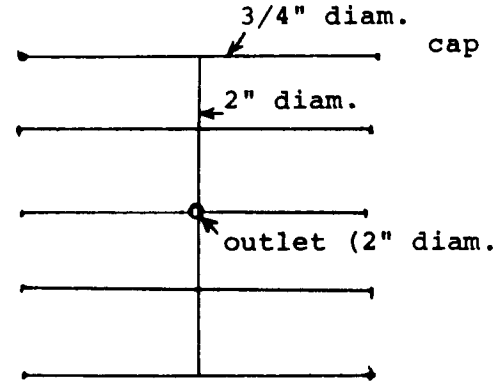


Fish Tanks with Filtering System



Sand Filter, Details
(side view)

Surface of sand in square feet should be approximately equal to the flow in gallons per minute



PVC Collector Pipes, Details
(viewed from above)

Each pipe has holes of 3/8" - 1/2" diam. spaced regularly at 2" intervals along length.

Figure 1. Diagram of Fish Tanks with Filtering System
(Details of Sand Filter and Collection Pipes Included)

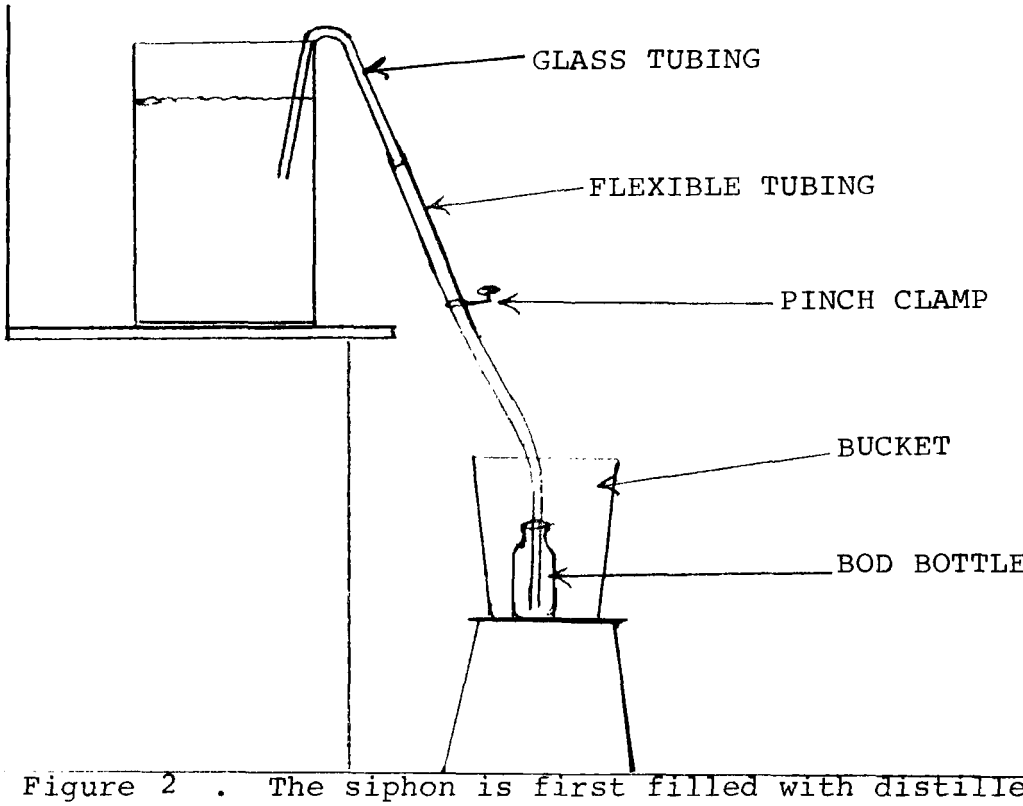


Figure 2 . The siphon is first filled with distilled water. After putting the glass tubing into the test water, the pinch clamp can be released and enough water siphoned into the bucket to displace the distilled water by test water. Then the end of the tube is put into the BOD bottle all the way to the bottom. After overflowing the bottle about twice, slowly withdraw the tubing, allowing the water to flow until the tube is out of the bottle. Start with the control water and proceed from the lower toxicant additions through the more concentrated ones. Then the siphon can be utilized without rinsing it with distilled water. Stopper the BOD bottles and measure the dissolved oxygen by the routine Winkler method.

Figure 3 - Data Recording Sheet

Source | Collection Date | Bioassay Date

Number and Kinds of Individuals | Temperature Range

Time	Parameter	Control		Waste Concentrations							
		Holding	Dilution								
0 hour	DO, mg/l										
	pH										
	EC, μmhos/cm										
	EDTA, as mg/l $CaCO_3$										
	JTU initial										
	1 hr										
24 hour	Survival										
	DO, mg/l										
48 hour	Survival										
	DO, mg/l										
72 hour	Survival										
	DO, mg/l										
96 hour	Survival										
	DO, mg/l										
	pH										
	EC, μmhos/cm										
	EDTA, as mg/l $CaCO_3$										
	JTU										

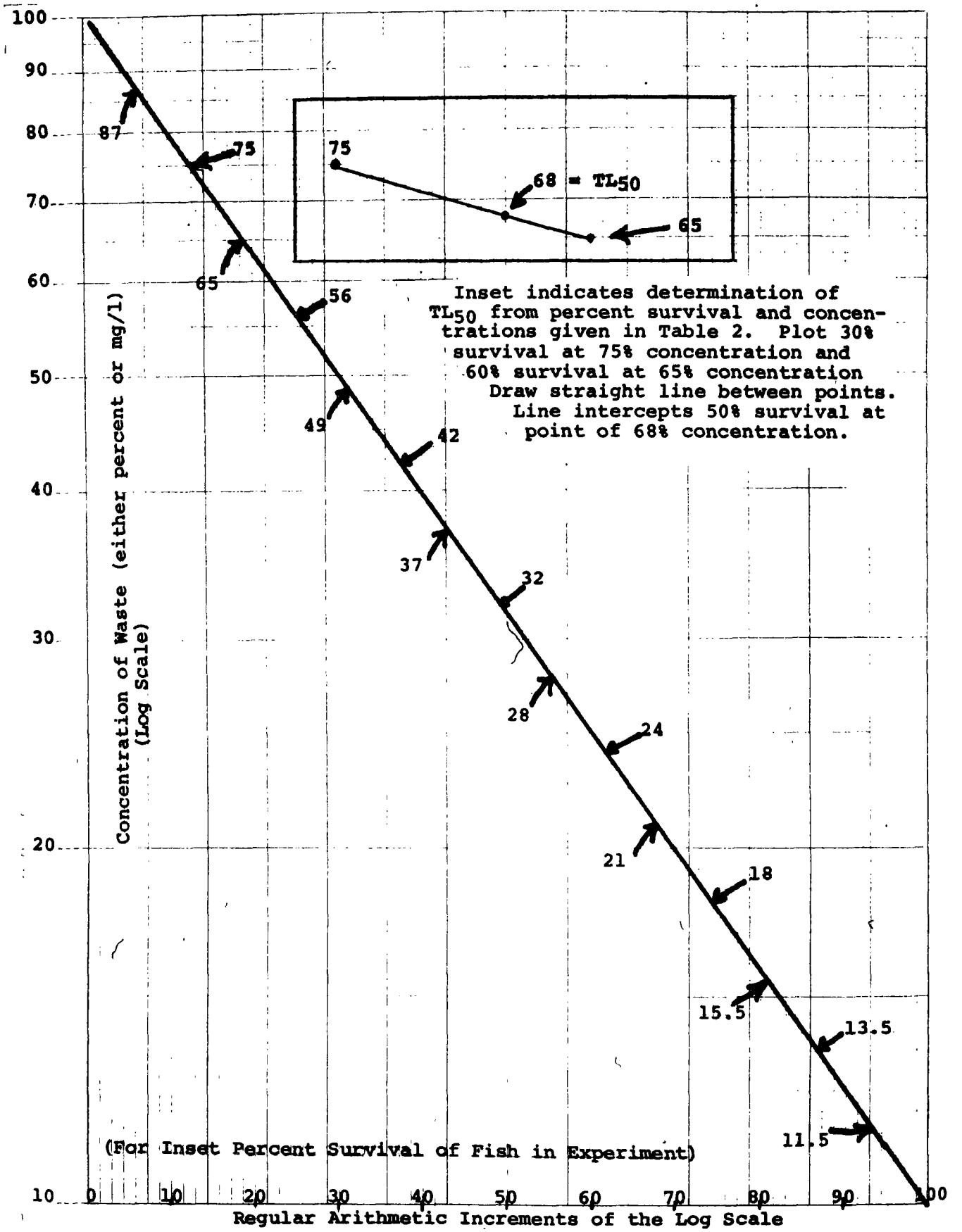


Figure 4. Guide to Fish Bioassay Concentration Selection

Figure 3 - Completed Data Form

Source Northwest STP Collection Date 5/25/72 Bioassay Date 5/25 - 5/29/72

Number and Kinds of Individuals 10 Stickleback / 15 liters Temperature Range 15.5 - 17.0 °C

96 hr TL_{50} = 46 % 95% Confidence limits = 40-52%

Effluent

Time	Parameter	Control		Waste Concentrations %						
		Holding	Dilution	18	32	42	56	65	75	100
0 hour	DO, mg/l	9.4	9.1	9.4	9.5	9.7	9.3	9.2	9.0	9.1
	pH	7.8	7.6	7.9	7.9	8.0	7.5	7.4	7.5	7.6
	EC, μmhos/cm	35000	35000	30000	26000	23000	19000	16000	12000	1700
	EDTA, as mg/l $CaCO_3$	7020	7040	5760	4680	3080	2480	2060	1460	208
	JTU initial	<5	<5	22	33	40	53	59	68	84
	1 hr									
24 hour	Survival	10	10	10	10	10	0	0	0	0
	DO, mg/l	7.2	9.0	8.7	9.7	9.0	9.5	8.2	8.3	8.4
48 hour	Survival	10	10	10	10	9				
	DO, mg/l	7.2	9.1	8.6	9.6	9.1				
72 hour	Survival	10	10	10	10	9				
	DO, mg/l	7.1	9.1	8.8	8.8	8.8				
96 hour	Survival	10	10	10	10	7	0	0	0	0
	DO, mg/l	7.0	9.2	8.8	8.4	7.9	—	—	—	—
	pH									
	EC, μmhos/cm									
	EDTA, as mg/l $CaCO_3$									
	JTU									

Figure 6

CALCULATIONS FOR FISH BIOASSAY STATISTICAL CONFIDENCE LIMITS

Dose	Log of Dose	No.	Number Dead	Number Alive	Accumulated Dead[1]	Accumulated Alive[1]	Total	Cumulated % Mort.[2]
18%	1.2553	10	0	10	0	27	27	0
32	1.5052	10	0	10	0	17	17	0
42	1.6232	10	3	7	3	7	10	30
56	1.7482	10	10	0	13	0	13	100
65	1.8129	10	10	0	23	0	23	100
75	1.8751	10	10	0	33	0	33	100
100	2.0000	10	10	0	43	0	43	100

(S.E.) Standard error = $\sqrt{\frac{0.79\ hR}{n}}$

h = interval between doses

R = interquartile range which is $TL_{75} - TL_{25}$. If either of these TL_{50} values are not found, use either $2(TL_{50} - TL_{25})$ or $2(TL_{75} - TL_{50})$ as the R value.

0.79 is a constant

n = number of organisms in each concentration (use mean number if variable)

$$h = \frac{0.2499 + 0.1180 + 0.1250 + 0.647 + 0.0622 + 0.1249}{6}$$

h = 0.1241

$$TL_{25} = 1.5052 + \frac{25}{30}^{3}(0.1180) = 1.6035 = 40.1\%$$

$$TL_{50} = 1.6232 + \frac{20}{70}(0.1250) = 1.6589 = 45.6\%$$

$$TL_{75} = 1.6232 + \frac{45}{70}(0.1250) = 1.7036 = 50.5\%$$

R = 1.7036 - 1.6035 = .1001

$$SE = \sqrt{\frac{0.79 \times 0.1241 \times .1001}{10}} = .0313$$

95% confidence limits equal:

$TL_{50} \pm 1.96$ (SE)

1.6589-1.96 (.0313) = 1.5976 = 39.5%

1.6589+1.96 (.0313) = 1.7202 = 52.5%

95% CL = 40% - 52%

1. Accumulative dead are derived from adding downwards in the numbers dead column and those alive by starting at the bottom of the numbers alive column and adding upwards.

2. Cummulative % Mortality = Accumulative dead ÷ total x 100

3. Interpolation to determine log value between 0 and 30% mortality.

VI. Use of Random Numbers

A. A table of random numbers is given in Table 1. This listing can be used in randomization processes needed for sample collection or experimental design.

For sample collection, the numbers selected would be used to pick the locations to be sampled. It would be essentially a process of limiting the number of sample points, all points having an equal probability of being selected.

In experiments the random numbers are used to assign positions of flasks, sequence of inoculation, etc. The uses of random tables for the two purposes will be explained below.

First it will be necessary to select the numbers from the table. This consists of (1) selecting the starting point and (2) listing sequentially a sufficient amount of numbers.

1. Selecting the starting point

Table 1 has the columns and rows each numbered 0-49. Without looking, put your eraser or finger-tip on any location in the table. Assume the point is at the intersection of column 20 and row 30. The numbers there are 4113. Then we can start using numbers at column 41 row 13. If the number selected is too high, just move along the row until a number under 50 is encountered or find another starting location.

2. Listing the numbers

Using the above location, write down the numbers. When getting to the end of the row start back in the reverse direction in the next lower

row. Group the numbers singly or in pairs depending on whether more or less than 10 samples have to be randomized.

Assume that we have 15 samples to put in random order, then starting at our above location we would have: 93 15 11 80 45 81 51 41 80 16 57 42 87 53 95 65 36 etc., continuing until we have encountered numbers 1-15. In actual practice we would not write down the numbers until one 15 or under was encountered.

B. Use of the tables in experiments

Fish bioassays

In these experiments, we would like at least to randomize the position of the jugs on the bench, and add 2 fish per jug in the randomized order. For example if we had the following jugs:

	Control	1%	10%	25%	50%	75%	100%
Given Numbers	1	2	3	4	5	6	7

Utilizing the single sequence of numbers above, we would have on the bench the jugs so:

10%	Control	50%	25%	75%	100%	1%
3	1	5	4	6	7	2

We could re-number the jugs as they appear now on the bench 1, 2, 3 --- and utilize a new selected sequence of numbers for adding 2 more fish per jug. However, time considerations would probably preclude this approach, although its advantage should not be overlooked in a completely randomized experiment.

Variations to the above approach, will probably be obvious.

C. Algal Bioassay Flasks

Randomization is possible in the sequence of adding algae, placement of flasks on shelves, mass measurement order, etc. The importance of randomization in bioassays probably should be secondary to an orderly sequence which would minimize errors.

Table 1

TEN THOUSAND RANDOM DIGITS

	00–04	05–09	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49
00	88758	66605	33843	43623	62774	25517	09560	41880	85126	60755
01	35661	42832	16240	77410	20686	26656	59698	86241	13152	49187
02	26335	03771	46115	88133	40721	06787	95962	60841	91788	86386
03	60826	74718	56527	29508	91975	13695	25215	72237	06337	73439
04	95044	99896	13763	31764	93970	60987	14692	71039	34165	21297
05	83746	47694	06143	42741	38338	97694	69300	99864	19641	15083
06	27998	42562	63402	10056	81668	48744	08400	83124	19896	18805
07	82685	32323	74625	14510	85927	28017	80588	14756	54937	76379
08	18386	13862	10988	04197	18770	72757	71418	81133	69503	44037
09	21717	13141	22707	68165	58440	19187	08421	23872	03036	34208
10	18446	83052	31842	08634	11887	86070	08464	20565	74390	36541
11	66027	75177	47398	66423	70160	16232	67343	36205	50036	59411
12	51420	96779	54309	87456	78967	79638	68869	49062	02196	55109
13	27045	62626	73159	91149	96509	44204	92237	29969	49315	11804
14	13094	17725	14103	00067	68843	63565	93578	24756	10814	15185
15	92382	62518	17752	53163	63852	44840	02592	88572	03107	90169
16	16215	50809	49326	77232	90155	69955	93892	70445	00906	57002
17	09342	14528	64727	71403	84156	34083	35613	35670	10549	07468
18	38148	79001	03509	79424	39625	73315	18811	86230	99682	82896
19	23689	19997	72382	15247	80205	58090	43804	94548	82693	22799
20	25407	37726	73099	51057	68733	75768	77991	72641	95386	70138
21	25349	69456	19693	85568	93876	18661	69018	10332	83137	88257
22	02322	77491	56095	03055	37738	18216	81781	32245	84081	18436
23	15072	33261	99219	43307	39239	79712	94753	41450	30944	53912
24	27002	31036	85278	74547	84809	36252	09373	69471	15606	77209
25	66181	83316	40386	54316	29505	86032	34563	93204	72973	90760
26	09779	01822	45537	13128	51128	82703	75350	25179	86104	40638
27	10791	07706	87481	26107	24857	27805	42710	63471	08804	23455
28	74833	55767	31312	76611	67389	04691	39687	13596	88730	86850
29	17583	24038	83701	28570	63561	00098	60784	76098	84217	34997
30	45601	46977	39325	09286	41133	34031	94867	11849	75171	57682
31	60683	33112	65995	64203	18070	65437	13624	90896	80945	71987
32	29956	81169	18877	15296	94368	16317	34239	03643	66081	12242
33	91713	84235	75296	69875	82414	05197	66596	13083	46278	73498
34	85704	86588	82837	67822	95963	83021	90732	32661	64751	83903
35	17921	26111	35373	86494	48266	01888	65735	05315	79328	13367
36	13929	71341	80488	89827	48277	07229	71953	16128	65074	28782
37	03248	18880	21667	01311	61806	80201	47889	83052	31029	06023
38	50583	17972	12690	00452	93766	16414	01212	27964	02766	28786
39	10636	46975	09449	45986	34672	46916	63881	83117	53947	95218
40	43896	41278	42205	10425	66560	59967	90139	73563	29875	79033
41	76714	80963	74907	16890	15492	27489	06067	22287	19760	13056
42	22393	46719	02083	62428	45177	57562	49243	31748	64278	05731
43	70942	92042	22776	47761	13503	16037	30875	80754	47491	96012
44	92011	60326	86346	26738	01983	04186	41388	03848	78354	14964
45	66456	00126	45685	67607	70796	04889	98128	13599	93710	23974
46	96292	44318	20898	02227	76512	53185	03057	61375	10760	26889
47	19680	07146	53951	10935	23333	76233	13706	20502	60405	09745
48	67347	51142	24536	60151	05498	64678	87569	65066	17790	55413
49	95888	59255	06898	99137	50871	81265	42223	83303	48694	81953

VII. APPENDIX

Contents

Equivalent Values

Depth

1 fathom = 6 feet
= 1.829 meters

Area

1 square inch = 6.42 square centimeters
1 square foot = 929.03 square centimeters
1 square yard = 0.836 square meter
1 acre = 43,560 square feet
= 4840 square yards
= 160 square rods
= 10 square chains (Gunter's)
= 0.4047 hectare
1 section = 640 acres
= 1 square mile
1 square mile = 640 acres
= 259 hectares
= 2.59 square kilometers
1 square millimeter = 0.0015 square inch
1 square meter = 10.758 square feet
1 hectare = 10,000 square meters
= 2.5 acres (approximately)

Volume

1 cubic inch = 16.386 cubic centimeters
1 cubic foot = 28,316 cubic centimeters
= 7.48 gallons
= 0.0283 cubic meter
1 cubic yard = 0.7646 cubic meter
1 acre-foot = 325,850 gallons
1,000,000 cubic feet = 22.95 acre-feet
1 cubic centimeter = 0.061 cubic inch
1 cubic meter = 35.314 cubic feet
= 1.308 cubic yards

Length

1 inch = 25.40 millimeters
= 2.54 centimeters
1 foot = 0.305 meter
= 30.5 centimeters
1 yard = 3 feet
= 0.914 meter
1 rod = 16.5 feet
= 5.5 yards
1 mile (statute) = 63,360 inches
= 5280 feet
= 1760 yards
= 320 rods
= 1609 meters
= 1.609 kilometers
= 0.867 geographic mile
1 millimeter = 0.0393 inch
1 centimeter = 0.393 inch
1 meter = 39.37 inches
= 3.281 feet
= 1.0936 yards
= 0.000621 mile
1 kilometer = 3281 feet
= 1000 meters
1 chain (Gunter's) = 792 inches
= 66 feet
= 4 rods
= 0.0125 mile
1 link (Gunter's) = 7.92 inches
= 0.04 rod
1 chain (engineer's) = 100 feet
1 link (engineer's) = 1 foot

Equivalent Values

Capacity

1 U.S. pint = 473.18 cubic centimeters
1 U.S. quart = 2 pints
= 946 cubic centimeters
= 0.946 liter
1 U.S. gallon = 231 cubic inches
= 4 quarts
= 3784 cubic centimeters
= 3.784 liters
1,000,000 gallons = 3.07 acre-feet
1 liter = 61.027 cubic inches
= 2.11 pints
= 1.0567 quarts
= 1000 cubic centimeters

Miscellaneous

1 atmosphere pressure = about 15 pounds per square inch
= about 1 ton per square foot
= about 1 kilo per square centimeter

Angles

1 circumference = 360 degrees
1 degree = 60 minutes
1 minute = 60 seconds

METRIC SYSTEM	ENGLISH SYSTEM
Units of Length	
Meter (m.) = 39.37 inches (in.)	Yard = 0.9144 m.
Centimeter (cm.) = 0.01 m.	Inch (U.S.) = 2.54 cm. (Fig. 1–5)
Millimeter (mm.) = 0.001 m.	
Kilometer (km.) = 1000 m.	Mile (U.S.) = 1.609 km.
Angstrom unit (A.U. or Å) = 10^{-8} cm.	
Units of Volume	
Liter (l.) = volume of 1 kg. of water	Liquid quart (U.S.) = 0.9463 l.
Milliliter (ml.) = 0.001 l.	Cubic foot (U.S.) = 28.316 l.
Units of Weight	
Gram (g.) = weight of 1 ml. of water at 4° C	Ounce (oz.)(avoirdupois) = 28.35 g.
Milligram (mg.) = 0.001 g.	Pound (lb.) (avoirdupois) = 0.4536 kg.
Kilogram (kg.) = 1000 g.	Ton (short) = 907.185 kg.
Ton (metric) = 1000 kg. = 2204.62 lb.	Ton (long) = 2240 lb. = 1.016 metric tons

NUMERICAL EQUIVALENTS

Length

1 in. = 2.540 cm
1 ft = 30.48 cm
1 mi = 1.609 km
1 cm = 0.3937 in.
1 m = 39.37 in.
1 km = 0.6214 mi
1 m = 3.28 ft

Speed

15 mi/hr = 22 ft/sec
1 mi/hr = 1.467 ft/sec
1 mi/hr = 44.7 cm/sec
1 km/hr = 27.78 cm/sec

Force

1 g-wt = 980 dynes
1 kg-wt = 2.205 lb
1 oz = 28.35 g-wt
1 lb = 453.6 g-wt
1 lb = 4.448×10^5 dynes
1 lb = 4.448 newtons
1 newton = 10^5 dynes
1 newton = 3.60 oz

Pressure

1 in. of mercury = 0.491 lb/in.^{2}
1 cm of mercury = 0.1934 lb/in.^{2}
1 cm of mercury = 0.0133 bar
1 ft of water = 0.433 lb/in.^{2}
1 in. of water = 0.0361 lb/in.^{2}
1 cm of water = 0.0142 lb/in.^{2}
1 cm of water = 0.980 millibar
1 lb/in.^{2} = 0.0690 bar
1 bar = 10^6 dynes/cm^2
1 bar = 14.5 lb/in.^{2}
1 atmosphere = 1.0132 bars
1 atmosphere = 14.7 lb/in.^{2}
1 atmosphere = 1.058 tons/ft^2
1 atmosphere = 76 cm of mercury

Work and Energy

1 joule = 10^7 ergs
1 joule = 0.738 ft-lb
1 joule = 0.000000278 kw-hr
1 joule = 0.000000373 hp-hr
1 joule = 0.239 cal
1 ft-lb = 1.35 joules
1 ft-lb = 1.35×10^7 ergs
1 ft-lb = 0.324 cal
1 ft-lb = 0.001286 Btu
1 cal = 4.18 joules
1 cal = 3.086 ft-lb
1 Btu = 252 cal
1 Btu = 778 ft-lb
1 Btu = 1055 joules
1 kw-hr = 3.6×10^6 joules
1 kw-hr = 2.655×10^6 ft-lb
1 kw-hr = 1.341 hp-hr
1 hp-hr = 1.98×10^6 ft-lb
1 hp-hr = 2.68×10^6 joules
1 hp-hr = 0.746 kw-hr

Power

1 hp = 746 watts
1 hp = 178 cal/sec
1 Btu/hr = 0.293 watts
1 kw = 1.34 hp
1 watt = 0.239 cal/sec

Electrical Quantities

10 amp = 1 em unit
10 coulombs = 1 em unit
1 coulomb = 3×10^9 es units
300 volts = 1 es unit
1 microfarad = 9×10^5 es units
1 millihenry = 10^6 em units

ACCEPTED VALUES OF CERTAIN QUANTITIES

Velocity of light in vacuo	299,776 km/sec
Gravitation constant	6.670×10^{-8} cgs unit
Electronic charge	4.80×10^{-10} es unit
Electronic charge	1.60×10^{-19} coul
Number of molecules at 0° C atmospheric pressure	2.69×10^{19} per cm^2
Number of molecules in 1 gram-molecular weight at 0° C atmospheric pressure (Avogadro's number)	6.0233×10^{23}
Mass of hydrogen atom	1.67×10^{-24} g
Mass of electron	9.11×10^{-28} g
Mass of electron in atomic mass units	5.486×10^{-4} amu
Mass of proton	1.67×10^{-24} g
Unit of atomic mass	1.660×10^{-24} g
Unit of atomic mass equivalent to	0.00146 erg
1 electron-volt	1.60×10^{-12} erg
Planck's constant (h)	6.624×10^{-27} erg-sec

SOME GEOMETRICAL RELATIONS

$\pi = 3.1416$, or $3\frac{1}{7}$ approximately

Circumference of a circle $= 2\pi r$

Area of a circle $= \pi r^2$

Area of a sphere $= 4\pi r^2$

Volume of a sphere $= \frac{4}{3}\pi r^3$

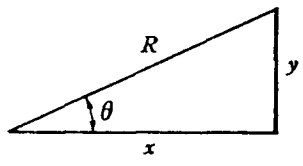
SOME TRIGONOMETRIC RELATIONS

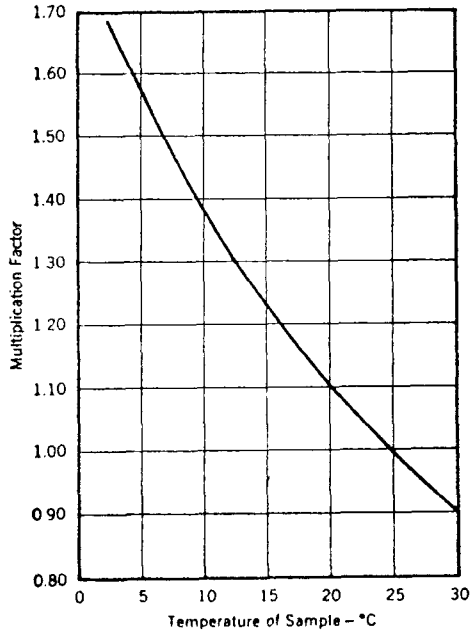
$\sin\theta = \frac{y}{R}$, or $y = R\sin\theta$.

$\cos\theta = \frac{x}{R}$, or $x = R\cos\theta$.

$\tan\theta = \frac{y}{x} = \frac{\sin\theta}{\cos\theta}$, or $y = x\tan\theta$.

$\cot\theta = \frac{x}{y} = \frac{\cos\theta}{\sin\theta}$, or $x = y\cot\theta$.





Factors for converting specific conductance of water to equivalent values at 25 C (based on 0.01*M* KCl solution).

Mathematical Formulas

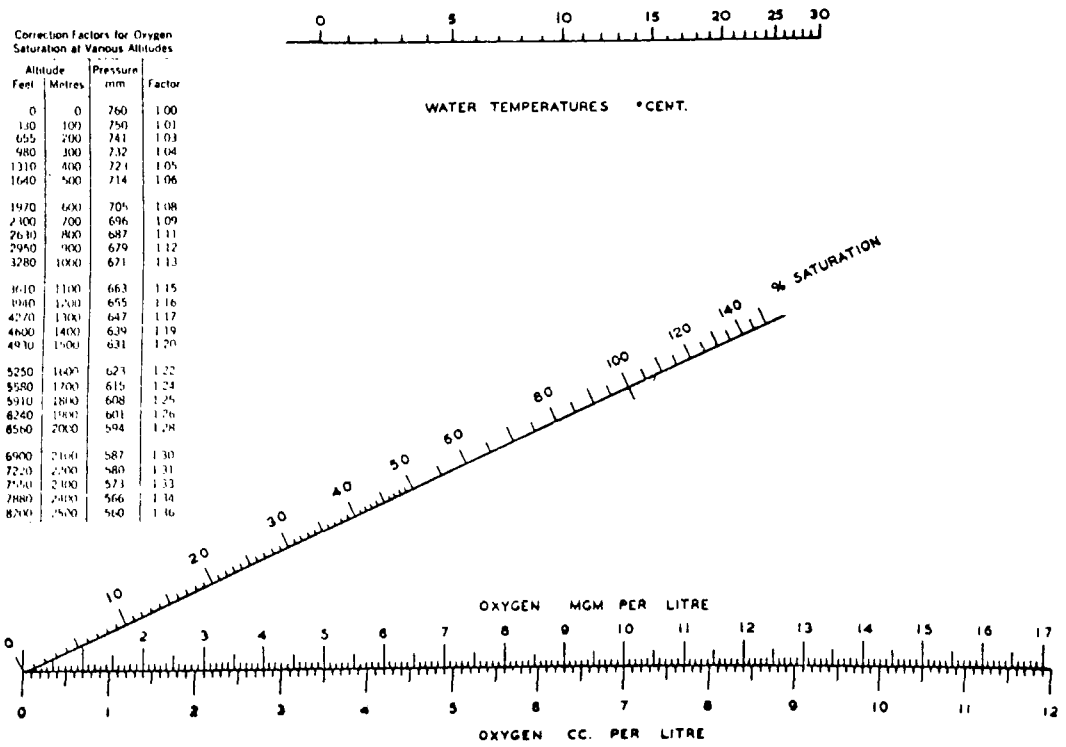
Given	*Sought*	*Formula*
Triangle		
1. Base (b) and altitude (a')	Area (a)	$a = \frac{ba'}{2}$, or $= b\left(\frac{a'}{2}\right)$
2. Area (a) and base (b) or altitude (a')	Base (b), or altitude (a')	$b = \frac{2a}{a'}$, or $a' = \frac{2a}{b}$
3. Three sides (d, d', d'')	Area (a)	Let s = sum of three sides, then $a = \sqrt{\left(\frac{s}{2}-d\right)\left(\frac{s}{2}-d'\right)\left(\frac{s}{2}-d''\right)\left(\frac{s}{2}\right)}$
4. Base (b) and perpendicular (p) of right-angle triangle	Hypotenuse (h)	$h = \sqrt{b^2+p^2}$
5. Base (b) or perpendicular (p) and hypotenuse (h) of right-angle triangle	Base (b), or perpendicular (p)	$b = \sqrt{h^2-p^2}$, or $p = \sqrt{h^2-b^2}$
Trapezoid		
6. Sides (s and s') and altitude (a')	Area (a)	$a = a'\left(\frac{s+s'}{2}\right)$
Trapezium		
7. Diagonal (d) and perpendiculars (p and p') to diagonal drawn from vertices of opposite angles	Area (a)	$a = d\left(\frac{p+p'}{2}\right)$
Circle		
8. Radius (r)	Circumference (c)	$c = 2\pi r$
9. Circumference (c)	Radius (r)	$r = \frac{2\pi}{c}$
10. Radius (r)	Area (a)	$a = \pi r^2$
Sphere		
11. Radius (r)	Surface (s)	$s = r^2(4\pi)$
12. Radius (r)	Volume (v)	$v = r^3\left(\frac{4\pi}{3}\right)$
Cylinder		
13. Radius (r) and altitude (a')	Convex surface (s), or volume (v)	$s = a'(2\pi r)$, or $v = a'(\pi r^2)$
Cone		
14. Radius (r) and altitude (a')	Volume (v)	$v = \frac{a'}{3}(\pi r^2)$
15. Radius (r) and slant height (b)	Convex surface (s)	$s = \frac{b}{2}(2\pi r)$
Frustrum of Cone		
16. Areas of both bases (b and b') and altitude (a')	Volume (v)	$v = \frac{a'}{3}(b+b'+\sqrt{bb'})$
17. Circumferences (c and c') and slant height (b)	Convex surface (s)	$s = \frac{b}{2}(c+c')$

Solubility of oxygen, from a wet atmosphere at a pressure of 760 mm. Hg, in mg. per liter, at temperatures from 0° to 35° C.

Temp.	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	14.16	14.12	14.08	14.04	14.00	13.97	13.93	13.89	13.85	13.81
1	13.77	13.74	13.70	13.66	13.63	13.59	13.55	13.51	13.48	13.44
2	13.40	13.37	13.33	13.30	13.26	13.22	13.19	13.15	13.12	13.08
3	13.05	13.01	12.98	12.94	12.91	12.87	12.84	12.81	12.77	12.74
4	12.70	12.67	12.64	12.60	12.57	12.54	12.51	12.47	12.44	12.41
5	12.37	12.34	12.31	12.28	12.25	12.22	12.18	12.15	12.12	12.09
6	12.06	12.03	12.00	11.97	11.94	11.91	11.88	11.85	11.82	11.79
7	11.76	11.73	11.70	11.67	11.64	11.61	11.58	11.55	11.52	11.50
8	11.47	11.44	11.41	11.38	11.36	11.33	11.30	11.27	11.25	11.22
9	11.19	11.16	11.14	11.11	11.08	11.06	11.03	11.00	10.98	10.95
10	10.92	10.90	10.87	10.85	10.82	10.80	10.77	10.75	10.72	10.70
11	10.67	10.65	10.62	10.60	10.57	10.55	10.53	10.50	10.48	10.45
12	10.43	10.40	10.38	10.36	10.34	10.31	10.29	10.27	10.24	10.22
13	10.20	10.17	10.15	10.13	10.11	10.09	10.06	10.04	10.02	10.00
14	9.98	9.95	9.93	9.91	9.89	9.87	9.85	9.83	9.81	9.78
15	9.76	9.74	9.72	9.70	9.68	9.66	9.64	9.62	9.60	9.58
16	9.56	9.54	9.52	9.50	9.48	9.46	9.45	9.43	9.41	9.39
17	9.37	9.35	9.33	9.31	9.30	9.28	9.26	9.24	9.22	9.20
18	9.18	9.17	9.15	9.13	9.12	9.10	9.08	9.06	9.04	9.03
19	9.01	8.99	8.98	8.96	8.94	8.93	8.91	8.89	8.88	8.86
20	8.84	8.83	8.81	8.79	8.78	8.76	8.75	8.73	8.71	8.70
21	8.68	8.67	8.65	8.64	8.62	8.61	8.59	8.58	8.56	8.55
22	8.53	8.52	8.50	8.49	8.47	8.46	8.44	8.43	8.41	8.40
23	8.38	8.37	8.36	8.34	8.33	8.32	8.30	8.29	8.27	8.26
24	8.25	8.23	8.22	8.21	8.19	8.18	8.17	8.15	8.14	8.13
25	8.11	8.10	8.09	8.07	8.06	8.05	8.04	8.02	8.01	8.00
26	7.99	7.97	7.96	7.95	7.94	7.92	7.91	7.90	7.89	7.88
27	7.86	7.85	7.84	7.83	7.82	7.81	7.79	7.78	7.77	7.76
28	7.75	7.74	7.72	7.71	7.70	7.69	7.68	7.67	7.66	7.65
29	7.64	7.62	7.61	7.60	7.59	7.58	7.57	7.56	7.55	7.54
30	7.53	7.52	7.51	7.50	7.48	7.47	7.46	7.45	7.44	7.43
31	7.42	7.41	7.40	7.39	7.38	7.37	7.36	7.35	7.34	7.33
32	7.32	7.31	7.30	7.29	7.28	7.27	7.26	7.25	7.24	7.23
33	7.22	7.21	7.20	7.20	7.19	7.18	7.17	7.16	7.15	7.14
34	7.13	7.12	7.11	7.10	7.09	7.08	7.07	7.06	7.05	7.05
35	7.04	7.03	7.02	7.01	7.00	6.99	6.98	6.97	6.96	6.95

Correction Factors for Oxygen Saturation at Various Altitudes

Altitude Feet	Altitude Metres	Pressure mm	Factor
0	0	760	1.00
330	100	750	1.01
655	200	741	1.03
980	300	732	1.04
1310	400	724	1.05
1640	500	714	1.06
1970	600	705	1.08
2300	700	696	1.09
2630	800	687	1.11
2950	900	679	1.12
3280	1000	671	1.13
3610	1100	663	1.15
3940	1200	655	1.16
4270	1300	647	1.17
4600	1400	639	1.19
4930	1500	631	1.20
5250	1600	623	1.22
5580	1700	615	1.23
5910	1800	608	1.25
6240	1900	601	1.26
6560	2000	594	1.28
6900	2100	587	1.30
7220	2200	580	1.31
7550	2300	573	1.33
7880	2400	566	1.34
8200	2500	560	1.36



Nomogram for determining O_2 saturation at different temperatures and altitudes.

Temperatures—Centigrade to Fahrenheit*

Temp. ° *C.*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
0	32.0	33.8	35.6	37.4	39.2	41.0	42.8	44.6	46.4	48.2
10	50.0	51.8	53.6	55.4	57.2	59.0	60.8	62.6	64.4	66.2
20	68.0	69.8	71.6	73.4	75.2	77.0	78.8	80.6	82.4	84.2
30	86.0	87.8	89.6	91.4	93.2	95.0	96.8	98.6	100.4	102.2
40	104.0	105.8	107.6	109.4	111.2	113.0	114.8	116.6	118.4	120.2
50	122.0	123.8	125.6	127.4	129.2	131.0	132.8	134.6	136.4	138.2

*Temperatures in degrees Centigrade expressed in left vertical column and in top horizontal row; corresponding temperatures in degrees Fahrenheit in body of table.

Temperatures—Fahrenheit to Centigrade*

Temp. ° *F.*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
30	− 1.11	− 0.56	0.00	0.56	1.11	1.67	2.22	2.78	3.33	3.89
40	4.44	5.00	5.56	6.11	6.67	7.72	7.78	8.33	8.89	9.44
50	10.00	10.56	11.11	11.67	12.22	12.78	13.33	13.89	14.44	15.00
60	15.56	16.11	16.67	17.22	17.78	18.33	18.89	19.44	20.00	20.56
70	21.11	21.67	22.22	22.78	23.33	23.89	24.44	25.00	25.56	26.11
80	26.67	27.22	27.78	28.33	28.89	29.44	30.00	30.56	31.11	31.67
90	32.22	32.78	33.33	33.89	34.44	35.00	35.56	36.11	36.67	37.22
100	37.78	38.33	38.89	39.44	40.00	40.56	41.11	41.67	42.22	42.78

*Temperatures in degrees Fahrenheit expressed in left vertical column and in top horizontal row; corresponding temperatures in degrees Centigrade in body of table.

Meters to Feet*

Meters	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
0	0.00	3.28	6.56	9.84	13.12	16.40	19.69	22.97	26.25	29.53
10	32.81	36.09	39.37	42.65	45.93	49.21	52.49	55.78	59.06	62.34
20	65.62	68.90	72.18	75.46	78.74	82.02	85.30	88.58	91.87	95.15
30	98.43	101.71	104.99	108.27	111.55	114.83	118.11	121.39	124.67	127.96
40	131.24	134.52	137.80	141.08	144.36	147.64	150.92	154.20	157.48	160.76
50	164.04	167.33	170.61	173.89	177.17	180.45	183.73	187.01	190.29	193.57
60	196.85	200.13	203.42	206.70	209.98	213.26	216.54	219.82	223.10	226.38
70	229.66	232.94	236.22	239.51	242.79	246.07	249.35	252.63	255.91	259.19
80	262.47	265.75	269.03	272.31	275.60	278.88	282.16	285.44	288.72	292.00
90	295.28	298.56	391.84	305.12	308.40	311.69	314.97	318.25	321.53	324.81
100	328.09	331.37	334.65	337.93	341.21	344.49	347.78	351.06	354.34	357.62

*Length in meters expressed in left vertical column and in top horizontal row; corresponding lengths in *feet* in body of table.

Feet to Meters*

Feet	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
0	0.000	0.305	0.610	0.914	1.219	1.524	1.829	2.134	2.438	2.743
10	3.048	3.353	3.658	3.962	4.267	4.572	4.877	5.182	5.486	5.791
20	6.036	6.401	6.706	7.010	7.315	7.620	7.925	8.229	8.534	8.839
30	9.144	9.449	9.753	10.058	10.363	10.668	10.972	11.277	11.582	11.887
40	12.192	12.496	12.801	13.106	13.411	13.716	14.020	14.325	14.630	14.935
50	15.239	15.544	15.849	16.154	16.459	16.763	17.068	17.373	17.678	17.983
60	18.287	18.592	18.897	19.202	19.507	19.811	20.116	20.421	20.726	21.031
70	21.335	21.640	21.945	22.250	22.555	22.859	23.164	23.469	23.774	24.079
80	24.383	24.688	24.993	25.298	25.602	25.907	26.212	26.517	26.822	27.126
90	27.431	27.736	28.041	28.346	28.651	28.955	29.260	29.565	29.870	30.174
100	30.479	30.784	31.089	31.394	31.698	32.003	32.308	32.613	32.918	33.222

*Length in feet expressed in left vertical column and in top horizontal row; corresponding lengths in *meters* in body of table.

AVERAGE APERTURE SIZE OF STANDARD GRADE DEFOUR BOLTING SILK

Silk No.	*Meshes per Inch*	*Size of Aperture (mm.)*	*Silk No.*	*Meshes per Inch*	*Size of Aperture (mm.)*
0000	18	1.364	10	109	0.158
000	23	1.024	11	116	0.145
00	29	0.752	12	125	0.119
0	38	0.569	13	129	0.112
1	48	0.417	14	139	0.099
2	54	0.366	15	150	0.094
3	58	0.333	16	157	0.086
4	62	0.318	17	163	0.081
5	66	0.282	18	166	0.079
6	74	0.239	20	173	0.076
7	82	0.224	21	178	0.069
8	86	0.203	25	200	0.064
9	97	0.168			

GRADES AND SIZE RANGES OF SILK BOLTING CLOTH

Grade	*Range of Sizes*
Standard	Nos. 0000–25
X quality	Nos. 6–17
XX quality	Nos. 0000–16
XXX quality	Nos. 6–18
Grit gauze	Nos. 14–72
XXX Grit gauze	Nos. 14–72

WENTWORTH'S CLASSIFICATION OF COARSER SEDIMENTS BASED UPON SIZE OF PARTICLES

Diameter of Particle in mm.	*Name Applied to Particle*
More than 256	Boulder
256–64	Cobble
64–4	Pebble
4–2	Granule
2–1	Very coarse sand
1–0.5	Coarse sand
0.5–0.25	Medium sand
0.25–0.125	Fine sand
0.125–0.062	Very fine sand
0.062–0.004	Silt
Less than 0.004	Clay

WENTWORTH GRADE SCALE, $\sqrt{2}$ SCALE, $\sqrt[4]{2}$ SCALE, CORRESPONDING TYLER SIEVE OPENINGS AND MESH, AND CORRESPONDING MESH OF U.S. SIEVE SERIES

Wentworth Grade Scale (mm.)	*The Openings Increase in the Ratio of*		*Tyler Screens*		*U.S. Sieve Series, Mesh*
	$\sqrt{2}$ *or 1.414 mm.*	$\sqrt[4]{2}$ *or 1.189 mm.*	*Mm.*	*Mesh*	
4	4.00	4.00	3.96	5	5
Granule		3.36	3.33	6	6
	2.83	2.83	2.79	7	7
		2.38	2.36	8	8
2	2.00	2.00	1.98	9	10
Very coarse sand		1.68	1.65	10	12
	1.41	1.41	1.40	12	14
		1.19	1.17	14	16
1	1.00	1.00	0.991	16	18
Coarse sand		0.840	0.833	20	20
	0.707	0.707	0.701	24	25
		0.595	0.589	28	30
0.500 (½)	0.500	0.500	0.495	32	35
Medium sand		0.420	0.417	35	40
	0.354	0.354	0.351	42	45
		0.297	0.295	48	50
0.250 (¼)	0.250	0.250	0.246	60	60
Fine sand		0.210	0.208	65	70
	0.177	0.177	0.175	80	80
		0.149	0.147	100	100
0.125 (⅛)	0.125	0.125	0.124	115	120
Very fine sand		0.105	0.104	150	140
	0.088	0.088	0.088	170	170
		0.074	0.074	200	200
0.062 ($\frac{1}{16}$)	0.062	0.062	0.061	250	230
Silt					

TABLE OF FORMULA WEIGHTS

$AgBr$	187.80
$AgBrO_3$	235.80
$AgCNS$	165.96
$AgCl$	143.34
Ag_2CrO_4	231.77
AgI	234.79
$AgIO_3$	282.79
$AgNO_3$	169.89
Ag_2O	231.76
Ag_3PO_4	418.62
Ag_2S	247.83
Al_2O_3	101.96
$Al(OH)_3$	78.00
$Al_2(SO_4)_3$	342.16
As_2O_3	197.82
As_2O_5	229.82
As_2S_3	246.02
$BaCO_3$	197.37
$Ba(CNS)_2$	253.53
$BaCl_2$	208.27
$Ba(ClO_4)_2$	336.27
$BaCrO_4$	253.37
BaO	153.36
BaO_2	169.36
$Ba(OH)_2$	171.38
$Ba_3(PO_4)_2$	602.03
$BaSO_4$	233.43
Bi_2S_3	514.20
$Ca_3(AsO_4)_2$	398.06
$CaBr_2$	199.91
$CaCO_3$	100.09
CaC_2O_4	128.10
CaF_2	78.08
$Ca(IO_3)_2$	389.90
CaO	56.08
$Ca(OH)_2$	74.10
$Ca_3(PO_4)_2$	310.19
$CaSO_4$	136.15
CeO_2	172.13
$Ce(SO_4)_2$	332.26
$H_4Ce(SO_4)_4$	528.42
$(NH_4)_2Ce(NO_3)_6$	548.26
$(NH_4)_2Ce(SO_4)_3 \cdot 2H_2O$	500.44
CO_2	44.01
$CO(NH_2)_2$ (urea)	60.06
Cr_2O_3	152.02
$CuCO_3$	123.55
CuI	190.45
CuO	79.54
Cu_2O	143.08
$CuSO_4 \cdot 5H_2O$	249.69
CuS	95.61
Cu_2S	159.15
$FeCO_3$	115.86
$Fe(CrO_2)_2$	223.87
FeO	71.85
Fe_2O_3	159.70
Fe_3O_4	231.55
$Fe(OH)_2$	89.87
$Fe(OH)_3$	106.87
FeS_2	119.98
$FeSO_4 \cdot 7H_2O$	278.03
$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$	392.16
$Fe_2(SO_4)_3$	399.90
HBr	80.92
$H_2C_2O_4 \cdot 2H_2O$ (oxalic)	126.07
$HC_2H_3O_2$ (acetic)	60.05
$HC_7H_5O_2$ (benzoic)	122.12
HCl	36.46
$HClO_4$	100.46
HNO_3	63.02
HNH_2SO_3 (sulfamic)	97.10
H_2O_2	34.02
H_3PO_4	98.00
H_2S	34.08
H_2SO_3	82.08
H_2SO_4	98.08
$Hg(NO_3)_2$	324.63
HgO	216.61
HgS	232.68
Hg_2Br_2	561.05
Hg_2Cl_2	472.13
Hg_2I_2	655.04
KBr	119.02
$KBrO_3$	167.02
KCN	65.12
$KCNS$	97.19
K_2CO_3	138.21
KCl	74.56
$KClO_3$	122.56
$KClO_4$	138.56
K_2CrO_4	194.21
$K_2Cr_2O_7$	294.22
$K_3Fe(CN)_6$	329.26
$K_4Fe(CN)_6$	368.36
KHC_2O_4	128.13
$KHC_2O_4 \cdot H_2C_2O_4 \cdot 2H_2O$	254.20
$KHC_4H_4O_6$ (tartrate)	188.18
$KHC_8H_4O_4$ (phthalate)	204.22
$KH(IO_3)_2$	389.93
KH_2PO_4	137.09
K_2HPO_4	175.18

KI	166.01
KIO_3	214.01
KIO_4	230.01
$KMnO_4$	158.03
KNO_2	85.11
KNO_3	101.11
K_2O	94.20
KOH	56.11
K_2PtCl_6	486.03
K_2SO_4	174.27
Li_2CO_3	73.89
LiCl	42.40
Li_2SO_4	109.95
$MgCO_3$	84.33
$Mg(ClO_4)_2$	223.23
$MgNH_4PO_4$	137.34
MgO	40.32
$Mg(OH)_2$	58.34
$Mg_2P_2O_7$	222.59
$MgSO_4$	120.39
MnO_2	86.94
Mn_2O_3	157.88
Mn_3O_4	228.82
$Mn(OH)_2$	88.96
$Mn_2P_2O_7$	283.83
Na_3AsO_3	191.88
$Na_2B_4O_7$	201.26
NaBr	102.91
$NaBrO_3$	150.91
$NaC_2H_3O_2$	82.03
NaCN	49.01
NaCNS	81.08
Na_2CO_3	106.00
$Na_2C_2O_4$	134.01
NaCl	58.45
NaClO	74.45
$NaClO_2$	90.45
$NaHCO_3$	84.02
NaI	149.90
$NaNO_3$	85.00
Na_2O	61.98
Na_2O_2	77.98
NaOH	40.00
Na_3PO_4	163.95
Na_2S	78.05
Na_2SO_3	126.05
Na_2SO_4	142.05
$Na_2S_2O_3 \cdot 5H_2O$	248.19
NH_3	17.03
N_2H_4	32.05
$(NH_4)_2C_2O_4$	124.10
NH_4Cl	53.50
NH_4NO_3	80.05
NH_4OH	35.05
$(NH_4)_3PO_4 \cdot 12MoO_3$	1876.50
$(NH_4)_2PtCl_6$	443.91
$(NH_4)_2SO_4$	132.15
P_2O_5	141.95
$PbCO_3$	267.22
PbC_2O_4	295.23
$PbCrO_4$	323.22
PbI_2	461.03
$Pb(IO_3)_2$	557.03
$PbMoO_4$	367.16
$Pb(NO_3)_2$	331.23
PbO	223.21
PbO_2	239.21
Pb_3O_4	685.63
$Pb_3(PO_4)_2$	811.58
$PbSO_4$	303.27
Sb_2O_3	291.52
Sb_2O_4	307.52
Sb_2O_5	323.52
Sb_2S_3	339.72
SiO_2	60.09
$SnCl_2$	189.61
SnO_2	150.70
SO_2	64.07
SO_3	80.07
$SrCO_3$	147.64
SrC_2O_4	175.65
SrO	103.63
$Sr_3(PO_4)_2$	452.84
$SrSO_4$	183.70
TiO_2	79.90
UF_6	352.07
UO_3	286.07
U_3O_8	842.21
V_2O_5	181.90
$ZnBr_2$	225.21
ZnO	81.38
$Zn_2P_2O_7$	304.71
ZnS	97.45
$ZnSO_4$	161.45
Water for Hydrates:	
1 H_2O	18.02
2 H_2O	36.03
3 H_2O	54.04
4 H_2O	72.06
5 H_2O	90.08
6 H_2O	108.10
7 H_2O	126.11

ATOMIC WEIGHTS

Element	Symbol	At. No	At.Wt.
Actinium	Ac	89	227
Aluminum	Al	13	26.98
Americium	Am	95	[243]
Antimony	Sb	51	121.76
Argon	A	18	39.944
Arsenic	As	33	74.91
Astatine	At	85	[210]
Barium	Ba	56	137.36
Berkelium	Bk	97	[245]
Beryllium	Be	4	9.013
Bismuth	Bi	83	209.00
Boron	B	5	10.82
Bromine	Br	35	79.916
Cadmium	Cd	48	112.41
Calcium	Ca	20	40.08
Californium	Cf	98	[248]
Carbon	C	6	12.011
Cerium	Ce	58	140.13
Cesium	Cs	55	132.91
Chlorine	Cl	17	35.457
Chromium	Cr	24	52.01
Cobalt	Co	27	58.94
Columbium (see Niobium)			
Copper	Cu	29	63.54
Curium	Cm	96	[245]
Dysprosium	Dy	66	162.51
Einsteinium	E	99	[254]
Erbium	Er	68	167.27
Europium	Eu	63	152.0
Fermium	Fm	100	[252]
Fluorine	F	9	19.00
Francium	Fr	87	[233]
Gadolinium	Gd	64	157.26
Gallium	Ga	31	69.72
Germanium	Ge	32	72.60
Gold	Au	79	197.0
Hafnium	Hf	72	178.50
Helium	He	2	4.003
Holmium	Ho	67	164.94
Hydrogen	H	1	1.0080
Indium	In	49	114.82
Iodine	I	53	126.91
Iridium	Ir	77	192.2
Iron	Fe	26	55.85
Krypton	Kr	36	83.80
Lanthanum	La	57	138.92
Lead	Pb	82	207.21
Lithium	Li	3	6.940
Lutetium	Lu	71	174.99
Magnesium	Mg	12	24.32
Manganese	Mn	25	54.94

Element	Symbol	At. No	At. Wt
Mendelevium	Mv	101	[256]
Mercury	Hg	80	200.61
Molybdenum	Mo	42	95.95
Neodymium	Nd	60	144.27
Neon	Ne	10	20.183
Neptunium	Np	93	[237]
Nickel	Ni	28	58.71
Niobium	Nb	41	92.91
Nitrogen	N	7	14.008
Osmium	Os	76	190.2
Oxygen	O	8	16.
Palladium	Pd	46	106.4
Phosphorus	P	15	30.975
Platinum	Pt	78	195.09
Plutonium	Pu	94	[242]
Polonium	Po	84	210.
Potassium	K	19	39.100
Praseodymium	Pr	59	140.92
Promethium	Pm	61	[145]
Protoactinium	Pa	91	231.
Radium	Ra	88	226.05
Radon	Rn	86	222.
Rhenium	Re	75	186.22
Rhodium	Rh	45	102.91
Rubidium	Rb	37	85.48
Ruthenium	Ru	44	101.1
Samarium	Sm	62	150.35
Scandium	Sc	21	44.96
Selenium	Se	34	78.96
Silicon	Si	14	28.09
Silver	Ag	47	107.880
Sodium	Na	11	22.991
Strontium	Sr	38	87.63
Sulfur	S	16	32.066
Tantalum	Ta	73	180.95
Technetium	Tc	43	[99]
Tellurium	Te	52	127.61
Terbium	Tb	65	158.93
Thallium	Tl	81	204.39
Thorium	Th	90	232.05
Thulium	Tm	69	168.94
Tin	Sn	50	118.70
Titanium	Ti	22	47.90
Tungsten	W	74	183.86
Uranium	U	92	238.07
Vanadium	V	23	50.95
Xenon	Xe	54	131.3
Ytterbium	Yb	70	173.04
Yttrium	Y	39	88.92
Zinc	Zn	30	65.38
Zirconium	Zr	40	91.22

RELATIVE HUMIDITY

Dry-Bulb Thermometer: Degrees, Fahrenheit	Difference between Dry-Bulb and Wet-Bulb Thermometers																	
	1°	2°	3°	4°	5°	6°	7°	8°	9°	10°	11°	12°	13°	14°	15°	16°	17°	18°
50	93	87	80	74	67	61	55	50	44	38	33	27	22	16	11	6	1	0
52	94	87	81	75	69	63	57	51	46	40	35	30	24	20	15	10	5	0
54	94	88	82	76	70	64	59	53	48	43	38	32	28	23	18	13	8	4
56	94	88	82	77	71	65	60	55	50	44	40	35	30	25	21	16	12	8
58	94	89	83	78	72	67	61	56	51	46	42	37	33	28	24	19	15	11
60	94	89	84	78	73	68	63	58	53	48	44	39	34	30	26	22	18	14
62	95	89	84	79	74	69	64	59	54	50	45	41	37	32	28	24	20	16
64	95	90	85	79	74	70	65	60	56	51	47	43	38	34	30	27	23	19
66	95	90	85	80	75	71	66	61	57	53	49	45	40	36	32	29	25	22
68	95	90	85	81	76	71	67	63	58	54	50	46	42	38	34	31	27	24
70	95	90	86	81	77	72	68	64	60	55	52	48	44	40	36	33	29	26
72	95	91	86	82	77	73	69	65	61	57	53	49	45	42	38	35	31	28
74	95	91	86	82	78	74	70	66	62	58	54	50	47	43	40	36	33	30
76	95	91	87	82	78	74	70	66	63	59	55	52	48	45	41	38	35	31
78	96	91	87	83	79	75	71	67	63	60	56	53	49	46	43	39	36	33
80	96	92	87	83	79	75	72	68	64	61	57	54	51	47	44	41	38	35
82	96	92	88	84	80	76	72	69	65	62	58	55	52	48	45	42	39	36
84	96	92	88	84	80	77	73	69	66	63	59	56	53	49	46	44	41	38
86	96	92	88	84	81	77	73	70	67	63	60	57	54	51	48	45	42	39
88	96	92	88	85	81	77	74	71	67	64	61	58	55	52	49	46	43	40
90	96	92	88	85	81	78	75	71	68	65	62	59	56	53	50	47	44	41
92	96	92	89	85	82	78	75	72	69	65	62	59	56	54	51	48	45	43
94	96	93	89	86	82	79	75	72	69	66	63	60	57	54	52	49	46	44
96	96	93	89	86	82	79	76	73	70	67	64	61	58	55	53	50	47	45

Stock Solutions of Cations (50 mg. of cation per ml.)

Group	*Ion*	*Formula of Salt*	*Grams per 100 ml. of Solution*
I	Ag^+	$AgNO_3$	8.0
	Pb^{++}	$Pb(NO_3)_2$	8.0
	Hg_2^{++}	$Hg_2(NO_3)_2$	7.0 (dissolve in 0.6 M HNO_3)
II	Pb^{++}	$Pb(NO_3)_2$	8.0
	Bi^{+++}	$Bi(NO_3)_3 \cdot 5\ H_2O$	11.5 (dissolve in 3 M HNO_3)
	Cu^{++}	$Cu(NO_3)_2 \cdot 3\ H_2O$	19.0
	Cd^{++}	$Cd(NO_3)_2 \cdot 4\ H_2O$	13.8
	Hg^{++}	$HgCl_2$	6.8
	As^{+++}	As_4O_6	3.3 (heat in 50 ml. of 12 M HCl, then add 50 ml. of water)
	Sb^{+++}	$SbCl_3$	9.5 (dissolve in 6 M HCl, and dilute with 2 M HCl)
	Sn^{++}	$SnCl_2 \cdot 2\ H_2O$	9.5 (dissolve in 50 ml. of 12 M HCl. Dilute to 100 ml. with water. Add a piece of tin metal)
	Sn^{++++}	$SnCl_4 \cdot 3\ H_2O$	13.3 (dissolve in 6 M HCl)
III	Co^{++}	$Co(NO_3)_2 \cdot 6\ H_2O$	24.7
	Ni^{++}	$Ni(NO_3)_2 \cdot 6\ H_2O$	24.8
	Mn^{++}	$Mn(NO_3)_2 \cdot 6\ H_2O$	26.2
	Fe^{+++}	$Fe(NO_3)_3 \cdot 9\ H_2O$	36.2
	Al^{+++}	$Al(NO_3)_3 \cdot 9\ H_2O$	69.5
	Cr^{+++}	$Cr(NO_3)_3$	23.0
	Zn^{++}	$Zn(NO_3)_2$	14.5
IV	Ba^{++}	$BaCl_2 \cdot 2\ H_2O$	8.9
	Sr^{++}	$Sr(NO_3)_2$	12.0
	Ca^{++}	$Ca(NO_3)_2 \cdot 4\ H_2O$	29.5
V	Mg^{++}	$Mg(NO_3)_2 \cdot 6\ H_2O$	52.8
	NH_4^+	NH_4NO_3	22.2
	Na^+	$NaNO_3$	18.5
	K^+	KNO_3	13.0

Composition of Commercial Acids and Bases

Acid or Base	*Specific Gravity*	*Percentage by Weight*	*Molarity*	*Normality*
Hydrochloric	1.19	38	12.4	12.4
Nitric	1.42	70	15.8	15.8
Sulfuric	1.84	95	17.8	35.6
Acetic	1.05	99	17.3	17.3
Aqueous ammonia	0.90	28	14.8	14.8

In chemistry we use the exponential method of expressing very large and very small numbers. These numbers are expressed as a product of two numbers. The first number of the product is called the digit term. This term is usually a number not less than 1 and not greater than 10. The second number of the product is called the exponential term and is written as 10 with an exponent. Some examples of the exponential method of expressing numbers are given below.

$$1000 = 1 \times 10^{3}$$
$$100 = 1 \times 10^{2}$$
$$10 = 1 \times 10^{1}$$
$$1 = 1 \times 10^{0}$$
$$0.1 = 1 \times 10^{-1}$$
$$0.01 = 1 \times 10^{-2}$$
$$0.001 = 1 \times 10^{-3}$$
$$2386 = 2.386 \times 1000 = 2.386 \times 10^{3}$$
$$0.123 = 1.23 \times .1 = 1.23 \times 10^{-1}$$

The power (exponent) of 10 is equal to the number of places the decimal is shifted to give the digit number. The exponential method is particularly useful as a shorthand for big numbers. For example, $1{,}230{,}000{,}000 = 1.23 \times 10^{9}$; and $0.000{,}000{,}000{,}36 = 3.6 \times 10^{-10}$.

1. *Addition of Exponentials.* Convert all the numbers to the same power of 10 and add the digit terms of the number.

EXAMPLE. Add 5×10^{-5} and 3×10^{-3}

SOLUTION. $3 \times 10^{-3} = 300 \times 10^{-5}$

$$(5 \times 10^{-5}) + (300 \times 10^{-5}) = 305 \times 10^{-5} = 3.05 \times 10^{-3}$$

2. *Subtraction of Exponentials.* Convert all the numbers to the same power of 10 and take the difference of the digit terms.

EXAMPLE. Subtract 4×10^{-7} from 5×10^{-6}

SOLUTION. $4 \times 10^{-7} = 0.4 \times 10^{-6}$

$$(5 \times 10^{-6}) - (0.4 \times 10^{-6}) = 4.6 \times 10^{-6}$$

3. *Multiplication of Exponentials.* Multiply the digit terms in the usual way and add algebraically the exponents of the exponential terms.

EXAMPLE. Multiply 4.2×10^{-8} by 2×10^{3}

SOLUTION.
$$\begin{array}{r} 4.2 \times 10^{-8} \\ \underline{2 \times 10^{3}} \\ 8.4 \times 10^{-5} \end{array}$$

4. *Division of Exponentials.* Divide the digit term of the numerator by the digit term of the denominator and subtract algebraically the exponents of the exponential terms.

EXAMPLE. Divide 3.6×10^{-5} by 6×10^{-4}

SOLUTION. $\dfrac{3.6 \times 10^{-5}}{6 \times 10^{-4}} = 0.6 \times 10^{-1} = 6 \times 10^{-2}$

5. *The Squaring of Exponentials.* Square the digit term in the usual way and multiply the exponent of the exponential term by 2.

EXAMPLE. Square the number 4×10^{-6}

SOLUTION. $(4 \times 10^{-6})^{2} = 16 \times 10^{-12} = 1.6 \times 10^{-11}$

6. *The Cubing of Exponentials.* Cube the digit term in the usual way and multiply the exponent of the exponential term by 3.

EXAMPLE. Cube the number 2×10^{3}

SOLUTION. $(2 \times 10^{3})^{3} = 2 \times 2 \times 2 \times 10^{9} = 8 \times 10^{9}$

7. *Extraction of Square Roots of Exponentials.* Decrease or increase the exponential term so that the power of ten is evenly divisible by 2. Extract the square root of the digit term by inspection or by logarithms and divide the exponential term by 2.

EXAMPLE. Extract the square root of 1.6×10^{-7}

SOLUTION. $1.6 \times 10^{-7} = 16 \times 10^{-8}$

$$\sqrt{16 \times 10^{-8}} = \sqrt{16} \times \sqrt{10^{-8}} = 4 \times 10^{-4}$$

Significant Figures

A bee keeper reports that he has 525,341 bees. The last three figures of the number are obviously inaccurate, for during the time the keeper was counting the bees, some of them would have died and others would have hatched; this would have made the exact number of bees quite difficult to determine. It would have been more accurate if he had reported the number 525,000. In other words, the last three figures are not significant, except to set the position of the decimal point. Their exact values have no meaning.

In reporting any information in terms of numbers, only as many significant figures should be used as are warranted by the accuracy of the measurement. The accuracy of measurements is dependent upon the sensitivity of the measuring instruments used. For example, if the weight of an object has been reported as 2.13 g., it is assumed that the last figure (3) has been estimated and that the weight lies between 2.125 g. and 2.135 g. The quantity 2.13 g. represents three significant figures. The weight of this same object as determined by a more sensitive balance may have been reported as 2.134 g. In this case one would assume the correct weight to be between 2.1335 g. and 2.1345 g., and the quantity 2.134 g. represents 4 significant figures. Note that the last figure is estimated and is also considered as a significant figure.

A zero in a number may or may not be significant, depending upon the manner in which it is used. When one or more zeros are used in locating a decimal point, they are not significant. For example, the numbers 0.063, 0.0063, and 0.00063, each have two significant figures. When zeros appear between digits in a number they are significant. For example, 1.008 g. has four significant figures. Likewise, the zero in 12.50 is significant. However, the quantity 1370 cm. has four significant figures provided the accuracy of the measurement includes the zero as a significant digit; if the digit 7 is estimated, then the number has only three significant figures.

The importance of significant figures lies in their application to fundamental computation. When adding or subtracting, the last digit that is retained in the sum or difference should correspond to the first doubtful decimal place (as indicated by underscoring).

EXAMPLE. Add 4.383 g. and 0.0023 g.

SOLUTION.
$$\begin{array}{r} 4.383\ \text{g.} \\ 0.0023 \\ \hline 4.385\ \text{g.} \end{array}$$

When multiplying or dividing, the product or quotient should contain no more digits than the least number of significant figures in the numbers involved in the computation.

EXAMPLE. Multiply 0.6238 by 6.6

SOLUTION. $0.6238 \times 6.6 = 4.1$

In rounding off numbers, increase the last digit retained by one if the following digit is five or more. Thus 26.5 becomes 27, and 26.4 becomes 26 in the rounding-off process.

The Use of Logarithms and Exponential Numbers

The common logarithm of a number is the power to which the number 10 must be raised to equal that number. For example, the logarithm of 100 is 2 because the number 10 must be raised to the second power to be equal to 100. Additional examples are as follows:

Number	*Number Expressed Exponentially*	*Logarithm*
10,000	10^4	4
1,000	10^3	3
10	10^1	1
1	10^0	0
0.1	10^{-1}	−1
0.01	10^{-2}	−2
0.001	10^{-3}	−3
0.0001	10^{-4}	−4

What is the logarithm of 60? Because 60 lies between 10 and 100, which have logarithms of 1 and 2, respectively, the logarithm of 60 must lie between 1 and 2. The logarithm of 60 is 1.7782, i.e., $60 = 10^{1.7782}$.

Every logarithm is made up of two parts, called the characteristic and the mantissa. The characteristic is that part of the logarithm which lies to the left of the decimal point; thus the characteristic of the logarithm of 60 is 1. The mantissa is that part of the logarithm which lies to the right of the decimal point; thus the mantissa of the logarithm of 60 is .7782. The characteristic of the logarithm of a number greater than 1 is one less than the number of digits to the left of the decimal point in the number.

Number	*Characteristic*	*Number*	*Characteristic*
60	1	2.340	0
600	2	23.40	1
6000	3	234.0	2
52840	4	2340.0	3

The mantissa of the logarithm of a number is found in the logarithm table (see Appendix B), and its value is independent of the position of the decimal point. Thus 2.340, 23.40, 234.0, and 2340.0 all have the same mantissa. The logarithm of 2.340 is 0.3692, that of 23.40 is 1.3692, that of 234.0 is 2.3692, and that of 2340.0 is 3.3692.

The meaning of the mantissa and characteristic can be better understood from a consideration of their relationship to exponential numbers. For example, 2340 may be written 2.34×10^3. The logarithm of (2.34×10^3) = the logarithm of 2.34 + the logarithm of 10^3. The logarithm of 2.34 is .3692 (mantissa) and the logarithm of 10^3 is 3 (characteristic). Thus the logarithm of 2340 = 3 + .3692, or 3.3692.

The logarithm of a number less than 1 has a negative value, and a convenient method of obtaining the logarithm of such a number is given below. For example, we may obtain the logarithm of .00231 as follows: When expressed exponentially, $.00234 = 2.34 \times 10^{-3}$. The logarithm of 2.34×10^{-3} = the logarithm of 2.31 + the logarithm of 10^{-3}. The logarithm of 2.31 is .3692 (mantissa) and the logarithm of 10^{-3} is -3 (characteristic). Thus the logarithm of .00231 = .3692 + (−3) = .3692 − 3 = −2.6208. The abbreviated form for the expression (.3692 − 3) is $\bar{3}.3692$. Note that only the characteristic has a negative value in the logarithm $\bar{3}.3692$, and that the mantissa is positive. The logarithm $\bar{3}.3692$ may also be written as 7.3692 − 10.

To multiply two numbers we add the logarithms of the numbers. For example, suppose we multiply 412 by 353.

Logarithm of 412	= 2.6149
Logarithm of 353	= 2.5478
Logarithm of product	= $\bar{5}.1627$

The number which corresponds to the logarithm 5.1627 is 145400 or 1.454×10^5. Thus 1.45×10^5 is the product of 412 and 353.

To divide two numbers we subtract the logarithms of the numbers. Suppose we divide 412 by 353.

Logarithm of 412	= 2.6149
Logarithm of 353	= 2.5478
Logarithm of quotient	= 0.0671

The number which corresponds to the logarithm 0.0671 is 1.17. Thus 412 divided by 353 is 1.17.

Suppose we multiply 5432 by 0.3124. Add the logarithm of 0.3124 to that of 5432.

Logarithm of 5432	= 3.7350
Logarithm of 0.3124	= $\bar{1}.4948$
Logarithm of the product	= 3.2298

The number which corresponds to the logarithm 3.2298 is 1697 or 1.697×10^3.

Let us divide 5432 by 0.3124. Subtract the logarithm of 0.3124 from that of 5432.

Logarithm of 5432	= 3.7350
Logarithm of 0.3124	= $\bar{1}.4948$
Logarithm of the quotient	= 4.2402

The number which corresponds to the logarithm 4.2402 is 17390 or 1.739×10^4.

The extraction of roots of numbers by means of logarithms is a simple procedure. For example, suppose we extract the cube root of 7235. The logarithm of $\sqrt[3]{7235}$ or $(7235)^{\frac{1}{3}}$ is equal to $\frac{1}{3}$ of the logarithm of 7235.

Logarithm of 7235	= 3.8594
$\frac{1}{3}$ of 3.8591	= 1.2865

The number which corresponds to the logarithm 1.2865 is 19.34. Thus, 19.31 is the cube root of 7235.

☆ GPO 790-323

ELEMENTS OF A QUALITY ASSURANCE PROGRAM

By

Kathleen Shimmin
EPA, Region IX
San Francisco CA

Presented at the Workshop on Sampling, Monitoring and Analysis of Water and Wastewater, March 6-12, 1974, Honolulu HI.

(These procedures are minimum efforts. Depending upon the purpose of the analysis, even more rigorous procedures may be warranted.)

ELEMENTS OF A QUALITY ASSURANCE PROGRAM

A. Procedures for any given laboratory - CHEMISTRY

I. Intralaboratory procedures

1. Use established procedures (St.Meth., ASTM, EPA - choice depends upon need)
   a. Choose proper procedure for given sample - taking note of interferences. etc.
   b. Choose procedure with sensitivity appropriate for need. Note sensitivity.
   c. Have written laboratory manual and use it.
   d. Note procedure used when data reported (if a choice exists in the laboratory manual).
2. Demonstrate that analyst is capable of analysis
   a. Give proper training if necessary
   b. Routinely run standard curves, unknowns, blanks.
   c. Periodically run reference samples (preferably prepared by an independent laboratory)
   d. Prepare quality control charts for each analyst and each analysis
      i) Precision
      ii) Accuracy
   e. Have analysts crosscheck each other's calculations and technique
3. Have established procedures for quality assurance for the data being produced
   a. Check precision by analyzing duplicates, at least one per ten samples
   b. Check accuracy by analyzing spikes, at least one per ten samples
4. Take appropriate steps to redo samples when quality control chart limits are exceeded
5. Maintain permanent records in bound volumes
6. Have established safety precautions and adhere to them
7. Have established procedures to assure the quality of equipment, reagents, glassware
   a. Regular servicing
   b. Conductivity checks on deionized, distilled waters
   c. Date reagents, chemicals, solutions. Store properly
8. Prepare and follow written sampling and handling procedures. Procedures should be in accordance with EPA guidelines.

II. Interlaboratory procedures

1. Periodically analyze samples split with another lab.
2. Participate in round-robin test evaluations
3. Maintain contact with other laboratories
4. Participate in Laboratory evaluation programs

B. Procedures for any given laboratory - BACTERIOLOGY

I. Intralaboratory procedures

1. Use established procedures specified for a given sample (St. Meth. or EPA)

a. Choose proper procedure for a given sample

b. When required to quantify bacterial levels, choose procedure with appropriate sensitivity

c. Have written laboratory manual

d. Note procedure used when data reported

2. Demonstrate that analyst is capable of analysis

a. Give proper training

b. Split samples with other analysts routinely

c. Have analysts crosscheck each other's calculations and procedures

3. Have established procedures for quality assurance for data being produced (Example - EPA)

a. Membrane filters - run in duplicate, at least 4 dilutions per medium per sample; periodically confirm selected colonies with MPN procedures

b. MPN - use 5 tubes per dilution, at least 3 dilutions
For a selected percentage of tests go through to Completed Test

c. Standard Plate Count - run in duplicate

d. Analyze controls (blanks, known spikes)

e. Report data which falls within statistically significant confidence range for given test

i. MF, total coliforms 20 - 80 colonies per plate
Fecal coliforms 20 - 60 colonies per plate
Fecal streptococci 20 - 100 colonies per plate

ii. Plate Count (100 mm diameter) 30 - 300 colonies per plate

Numbers outside range should be reported "less than" or "greater than" and should be redone if necessary

4. Have established procedures to assure quality of media, cultures, glassware, equipment, etc. Maintain records of this.

a. Media

i. Note dates received, opened

ii. Discard outdated material (or use only for screening purposes)

iii. Store under proper conditions (temperature, humidity, light)

iv. Prepare media properly

v. Establish and maintain program to check media periodically (by batch lots) to assure appropriate positive and negative resul

b. Stock cultures (if these are maintained in laboratory)

i. Transfer at appropriate frequency (e.g., once per month)

ii. Routinely check purity of cultures by making streak plates, and repurify if necessary

c. Water supply system (distilled), for media preparation

i. Periodically check toxicity of distilled water to a given bacterial culture - usually Enterobacter aerogenes
Standard Methods recommends a frequency of at least once per yea

d. Equipment - repair

i. Maintain routine service contracts on major equipment such as autoclaves, microscopes, balances, hoods

ii. Keep other equipment in good repair - either by contract or through other means

iii. Note dates of service by placing labels on the equipment (labels should list date, name, and address of service person)

e. Equipment - performance

i. Use recording charts for temperature of waterbath, autoclave
Store these as lab records

ii. Record temperatures periodically for incubators - frequency depends upon usage
As an alternative, use a max-min thermometer and record results

iii. Test accuracy of automatic pipetting machine before and during use

iv. Test accuracy of thermometers against NBS-registered thermometer (with chart), appropriate for the desired temperature range

5. Maintain permanent records in bound volumes

6. Establish safety precautions and adhere to them

a. Sterilize all contaminated material before washing or discarding it

b. Use aseptic technique

c. Properly train all individuals before allowing them to work with potentially-contaminated material

d. Immunize lab workers against tetanus (and possibly typhoid or other disease as appropriate)

e. Etc.

7. Follow established sample handling procedures, which are in agreement with EPA guidelines

a. Adhere to temperature-of-storage conditions

b. Do not exceed maximum allowable period for time elapsing between collection and processing of sample

c. Establish chain-of-custody routine for possible enforcement samples

II. Interlaboratory procedures

1. Periodically analyze samples split with another lab

2. Participate in round-robin test evaluations

3. Maintain contact with other laboratories

4. Participate in laboratory evaluation programs

C. Procedures for any given laboratory - BIOASSAY

I. Intralaboratory procedures

1. Use established procedures
   a. Choose proper procedure and test animal for given sample
   b. Hold animal for required time under required conditions prior to initiating test
   c. Choose appropriate dilutions for documentations of standards compliance
   d. Have written laboratory manual
   e. Note procedure used when data reported
2. Demonstrate that analyst is capable of analysis
   a. Give proper training
   b. During learning period split samples with other analysts
3. Have established procedures for quality assurance for data being produced
   a. Statistically evaluate results
   b. Report confidence intervals for data, unless only an estimated figure is requested
4. Maintain permanent records in bound volumes
5. Have established safety precautions and adhere to them
6. Have established procedures to assure quality of equipment, reagents, glassware
7. Follow established sample handling procedures in accordance with EPA guidelines

II. Interlaboratory procedures

1. Periodically analyze samples split with another lab
2. Participate in round-robin test evaluations
3. Maintain contact with other laboratories
4. Participate in laboratory evaluation programs

ENVIRONMENTAL PROTECTION AGENCY

STANDARD METHODS

USED BY

REGION IX

MICROBIOLOGY LABORATORY

Region IX

Activities of the Microbiology Section

The Microbiology Laboratory has established tests which it can perform. These include: indicator organisms assays (total and fecal coliform, fecal streptococci) by multiple tube dilution and membrane filter techniques; plate counts at 20° and 35°C; pathogen isolation (Salmonella), serology, and fluorescent-antibody scanning. Methodology is attached.

Upon request the Section can adapt existing techniques and develop special ones for recovering such organisms as: anaerobic bacteria; photosynthetic bacteria; Pseudomonas sp.; sulfur oxidizers; Klebsiella; and other specific groups.

Staff from the Section will also offer technical advice and consultation for review of grants, permits, standards and research proposals, etc. Lectures and training courses in laboratory and field techniques and sample collection have been given by the Section in the past and can be developed or modified upon request.

Kathleen G. Shimmin
Chief, Microbiology Section

TABLE OF CONTENTS

TABLES

*Refers to Section Numbers in Standard Methods, 13th Edition.

STANDARD PROCEDURES USED BY REGION IX LABORATORY FOR BACTERIOLOGICAL EXAMINATION OF TOTAL AND FECAL COLIFORMS

Sample Collection and Chain-of-Custody

a. Procedure for collection of samples shall be done in accordance with methods as outlined in Standard Methods for the Examination of Water and Wastewater; 13th Edition.

b. Samples should be properly iced immediately after collection (ice bucket or chest).

c. Chain-of-custody should be as follows:

1. Two people are required; one as sampler, one as witness.

2. Label on sample container should read: (See Attachment - page 3)

   Sample Number
   Sample Description
   Time and Date Collected
   Collected by
   Witnessed by

   (Use a self-sticking label which will not deteriorate in ice chest)

3. If samples change hands during transport back to laboratory, label or tag should be attached to ice bucket or chest, signed by person and witness to whom custody was given. (See Attachment)

4. Upon delivery to laboratory the technican should make out a receipt stating time and condition samples were received.

5. Before processing, log in time received, time collected, time processed, and processor. This can be done in same log book as results are recorded.

Processing

a. Do at least duplicate replicate plates for each dilution.

b. For unknown water, do at least four dilutions with at least two replicates for each dilution. (A minimum of eight plates per medium per sample.)

3. Time lapse between sample collection and processing is as follows:

a.	Seawater	4 hours for Total Coliforms 2 hours for Fecal Coliforms
b.	Freshwater	6 hours for Total Coliforms 3-4 hours for Fecal Coliforms
c.	Shellfish	6 hours for Total and Fecal 12 hours maximum

Examination of shellfish for total and fecal coliforms should be done according to the procedures recommended in most recent edition of American Public Health Association, Recommended Procedures for the Examination of Seawater and Shellfish.

ATTACHMENT

Sample Description

Date **Time**

Sampler

Witness

SAMPLE BOTTLE LABEL

ENVIRONMENTAL PROTECTION AGENCY	SAMPLE NO.	SEAL BROKEN BY	DATE
	SIGNATURE		
	PRINT NAME AND TITLE *(Inspector, Analyst or Technician)*		

EPA Form 7500-2 (10-71)

CHAIN OF CUSTODY LABEL

RECEIPT OF SAMPLE

I hereby certify that I received this sample and disposed of it as noted below.

RECEIVED FROM	DATE RECEIVED	TIME RECEIVED

DISPOSITION OF SAMPLE	SIGNATURE

DISPATCH OF SAMPLE

I hereby certify that I obtained this sample and dispatched it as shown below.

DATE OBTAINED	TIME OBTAINED	SOURCE

DATE DISPATCHED	TIME DISPATCHED	METHOD OF SHIPMENT

SENT TO	SIGNATURE

CHAIN OF CUSTODY TAG

Standard Method used
by
Region IX, Laboratory

THE DETERMINATION OF COLIFORM ORGANISMS BY THE MEMBRANE FILTER PROCEDURE

M-ENDO - Total Coliforms

I. Preparation of Medium

1. Boil 500 ml. distilled water.

2. Add 1.5% Bacto-Agar (7.5 g) and stir to dissolve.

3. Add 24 g. Difco M-Endo medium and 10 ml of 95% ethanol. Bring to boil to dissolve (keep stirring).

   DO NOT CONTINUE TO BOIL the medium once boiling point reached.

4. Cool medium slightly and distribute about 5 ml per petri dish (sterile, disposable, 50 x 12 mm).

5. Allow the plates to harden. Pack the plates in an inverted position in a basket and cover with brown paper. Place the basket in the refrigerator.

   In the dark and under cool conditions the prepared medium can be stored for short periods of time. The prepared plates should be used within a 24 hour period Under no circumstances will the plates be stored for periods of 72 hours or longer. Results from such plates are questionable.

II. Testing Procedures

1. All filtrations should be carried out according to the protocol outlined in Section 408, page 678 of Standard Methods, 13th Edition, 1971.

2. Sample volumes to be filtered should be chosen so that at least one membrane filter contains between 20-80 coliform colonies, and not to exceed 200 colonies of all types on the filter.

In the absence of previous bacterial data the following are recommended volumes:

a.	Treated water supplies ----	minimum of 50 ml, 100 ml recommended.
b.	Untreated water supplies --	5 - 50 ml.
c.	Unpolluted surface water --	1, 4, 15 and 60 ml (covers range 33-8000).
d.	Polluted surface water ----	0.02, 0.08, 0.15 and 0.5 ml (covers range of 4000 - 40,000).
e.	Sewage ----	0.0003, 0.001, 0.003 and 0.01 ml (covers range of 200,000 - 27,000,000 cells/100ml).

3. The plates containing the membranes are placed in a 35°C incubator in an inverted position.

4. After a period of 24 $\pm$ 2 hours the plates are removed from the incubator. Coliform colonies are dark red and have a green metallic surface sheen. Non-coliform colonies range from colorless to pink, however, the metallic sheen is absent. Such type colonies should not be included in the coliform count.

5. The characteristic metallic sheen colonies are counted with the aid of a wide-field binocular microscope using 10 or 15 x magnification. For illumination, use a light source directly over the membrane filter so that an image of the light source is reflected off the colony surface into the microscope lens system. (Suitable for this purpose is a Stereozoom Microscope [A/O or B&L] with a fluorescent illuminator).

6. Select the membranes that have between 20 to 80 coliform colonies and compute the density per 100 ml. The actual colony counts and the calculated density per 100 ml are entered on the data sheet.

$$\frac{\text{No. of coliform colonies counted}}{\text{Sample volume filtered in ml}} \times 100 = \text{No. of colonies per 100 ml}$$

7. The plates are then placed in metal containers and sterilized in the autoclave. At no time will any culture medium containing bacteria be disposed of first without adequate sterilization.

8. Calculations

a. For routine work, at least 3 dilutions of the sample are made. However, when testing water where no previous information is available then it may be necessary to use as many as 4 or 5 dilutions.

b. Select the membrane that has between 20-80 coliform colonies and compute the density per 100 ml.

c. If several sample volumes have coliform colonies in the range 20-80, then average the counts per 100 ml.

d. If all membranes have counts outside the range 20-80, the following procedure should be used:

1. Low counts (below 20 colonies). Calculate the density if 20 colonies were to have been present. Report on this calculation on the data sheet preceded by "<" ("less than").

2, High counts (above 80 colonies). Calculate the density if 80 colonies were to have been present. Report this calculation on the data sheet preceded by ">" ("greater than").

It must be realized that data reported with "less than" and "greater than" have limited use when strict interpretation of data is required. It does provide some idea as to the relative coliform density of the sample. Most important of all, it shows the need for repeat sampling and adjustment of sample filtration volume so that a membrane with the desired range 20-80 may be obtained.

In order to obtain at least one membrane having an acceptable number of colonies, the range of filtration volumes should vary by a factor of 4 or less. Different factors apply to fecal coliform and fecal streptococcus.

REGION IX

THE DETERMINATION OF FECAL COLIFORM ORGANISMS BY THE MEMBRANE FILTER TECHNIQUE

I. Medium Preparation

1. Rehydrate Bacto-M-FC Broth Base by adding 3.7g in 100 ml distilled water.

2. Add one ml of a rosolic acid solution prepared by dissolving one gram rosolic acid in 100 ml 0.2N NaOh.

3. Add 1.5% Bacto Agar.

4. Dissolve ingredients in a boiling water bath or in "Instatherm" apparatus.

5. Bring to a boil and pour approximately 5 ml into sterile, disposable 50 x 12mm petri dishes. Final reaction of the medium is pH 7.4.

6. Allow the medium to harden.

7. Pack the plates in baskets in the inverted position and place in the refrigerator.

8. Prepared medium "shelf life" is 5-7 days if stored in the refrigerator away from light.

9. The rosolic acid is stable indefinitely in the dry state. In aqueous solution it is stable for two weeks under refrigeration. After this period degradation is evidenced by the change of color (Red to Brown).

II. Testing Procedures

1. All filtrations should be carried out according to the protocol outlined in Section 408, page 678 of Standard Methods, 13th Edition, 1971.

2. Sample volumes to be filtered should be chosen so that at least one membrane filter contains between 20-60 fecal coliform colonies.

3. The plates containing the membranes are placed in water-proof plastic bags (Whirl-Pak Bags) and submerged in a 44.5 ± 0.2°C water bath for 24 hours. (Plates should not be held no longer than 20 minutes at ambient temperature after filtration. Immediate introduction of plates into the 44.5°C water bath is recommended).

4. After incubation, colony counts are made using a wide-field binocular microscope - 10x magnification. Fecal coliform colonies are deep blue in color and may vary from one to three mm in diameter. Non-fecal coliform colonies will appear as pink or colorless type colonies and should not be included in the fecal coliform count.

5. The colony counts are entered on the data sheet and reported per 100 ml.

$$\frac{\text{No. of fecal coliform colonies counted}}{\text{Sample volume filtered in ml}} \times 100 = \text{No. of colonies per 100 ml.}$$

6. Calculations and selection of sample filtration volumes follow the same theory as that discussed in the Method, Total Coliform Determination by Membrane Filter Procedure. Exceptions are that the desired range of fecal coliform colonies on the membrane is 20-60, and that fecal coliform counts should be based on filtration volumes varying by a factor of 3 or less.

7. The plates are than placed in metal containers and sterilized in autoclave. At no time will any culture medium containing bacteria be disposed of first without adequate sterilization.

THE DETERMINATION OF FECAL STREPTOCOCCI BY THE MEMBRANE FILTER PROCEDURE

I. Preparation of Medium

1. Weigh out 21 grams of M-Enterococcus Agar and place in one liter flask.

2. Add 500 ml cold distilled water and bring solution to a boil using "Instatherm" or boiling water bath and remove as soon as agar is in solution. (Do not overheat.)

3. Final pH should be: 7.2

4. Pour approximately 5 ml of the medium into sterile, disposable, 50 x 12 mm petri dishes.

5. Allow the medium to harden. Packthe plates in an inverted position in a basket and cover with brown paper. The prepared plates can be stored in the refrigerator up to four weeks.

6. The filtration of the sample should be carried out according to the methods outlined in the section on Filtering Techniques.

7. Sample volume to be filtered should be chosen so that at least one membrane filter contains between 20-100 fecal streptococcus colonies.

8. The plates containing the membranes are placed in a 35°C incubator in an inverted position.

9. After a period of 48 hours the plates are removed from the incubator. With a wide-field binocular microscope, using 10 or 15x magnification, count all pink, red and purplish-red colonies. Colonies other than these are not fecal streptococci and should not be included in the count.

10. The colony counts are entered on the data sheet and reported per 100 ml.

$$\frac{\text{No. of fecal streptococci counted}}{\text{Sample volume filtered in ml}} \times 100 = \text{No. of fecal streptococci per 100 ml}$$

11. Calculation and selection of sample filtration volumes follow the same principle as that discussed in Method of Coliform Determination By Membrane Filter Procedure, except that the desired range of colonies on the membrane is between 20-100 and the counts should be based on filtration volumes varying by a factor of 5 or less.

12. The plates are than placed in metal containers and sterilized in the autoclave and discarded.

Standard Methods used by Region IX for the Determination of Fecal Streptococcus by Membrane Filter using Bacto-KF Streptococcus Agar

I. Preparation of Medium

a. Weigh out 76.4 grams KF Streptococcus agar and place in flask.

b. Add 1000 ml cold distilled water and heat to boiling to dissolve completely.

c. Dispense in 100 ml amounts or multiples thereof into flasks and sterilize for 10 minutes at 15 lb pressure (121°C).

d. Cool to 60°C and add one ml Bacto TTC Solution 1%. (Triphenyltetrazolium Chloride 1%) per 100 ml sterile medium.

e. Mix to obtain uniform distibution of the TTC.

f. Final pH 7.2.

g. Pour approximately 5 ml of medium into sterile disposable, 50x12 mm petri dishes.

h. Incubate inoculated plates for 48 hours at 35°C.

i. Using a dissecting microscope with a magnification of 15 diameters count all colonies showing red or pink center as streptococcus.

j. Colony counts are entered on data sheet and reported per 100 ml.

k. Calculation:

$$\frac{\text{Number of fecal streptococcus counted}}{\text{Sample volume filtered in ml}} \times 100 = \text{fecal streptococcus/100m}$$

l. Desired range of colonies on membrane is 20-100 and counts should be based on filtration volumes varying by a factor of 5 or less.

m. Plates are then placed in metal containers and sterilized in an autoclave. (120°C, 15 lb pressure for 1/2 hour).

THE MOST PROBABLE NUMBER (MPN) TEST FOR THE DETECTION AND ENUMERATION OF COLIFORM AND FECAL COLIFORM ORGANISMS

I. Presumptive Test

A. Preparation of medium

1. For 1 ml of sample inoculation: weigh out 35.6g lauryl tryptose broth and add to one liter of distilled water. (For 10 ml of sample inoculation 53.4g per one liter of distilled water).

2. Dissolve ingredients and dispense ten ml of mixture 1 above into test tubes. Dispense 20 ml of 53.4g per one liter mixture into large test tubes.

3. Insert one Durham gas tube, inverted position, into each tube containing the broth. Place autoclavable plastic Kaputs on tubes. The double-strength tubes may be coded by closing them with Kaputs of a different color.
4. Sterilize in the autoclave for 12 minutes at 12 lbs. pressure.

B. Procedure

1. The five tube, multiple fermentation technique will be used.

2. Inoculate a series of five tubes in each dilution. In all such analyses, at least 3 dilutions must be used. (Region IX routinely uses 5 dilutions.)

3. The portions of the water sample used for inoculating the fermentation tubes will vary with the type water being tested. In general, decimal multiples and sub-multiples of one ml will be used.

4. Incubate the fermentation tubes at 35.0 $\pm$ 0.5°C. Examine each tube at the end of 24 $\pm$ 2 hours and, if no gas had yet been produced, examine again at the end of 48 hours $\pm$ three hours.

5. Record the presence or absence of gas formation at each examination of the tubes. The smallest type bubble in the gas tube should be recorded as a positive tube, even though this may appear as trapped air in the tube and not really gas production from the fermentation process.

II. Confirmed Test

A. Preparation of medium

1. Weigh out 40.0g of Bacto-Brilliant Green Bile 2% and add to one liter of distilled water.

2. Dissolve ingredients and dispense ten ml into each test tube.

3. In the inverted position, insert a Durham gas vial into each test tube. Use Kaput closures to cover the tubes.

4. Sterilize in the autoclave for 12 minutes at 12 lbs. pressure. Final reaction of the medium is pH 7.2.

B. Procedure

*1. Using a 24 gauge wire loop, with a loop of 3 mm in diameter, transfer one loopful of the positive, lauryl tryptose broth into a tube of brilliant green bile broth.

(If active fermentation appears in the primary fermentation tube before the expiration of the 24 hour period of incubation, it is preferable to transfer to the confirmatory brilliant green bile broth without waiting for the full 24 hour period to elapse.)

2. Incubate the inoculated brilliant green lactose bile broth tube for 48 $\pm$ 3 hours at 35° $\pm$ 0.5°C.

3. The formation of gas in any amount in the Durham tube, constitutes a positive Confirmed Test. Record all positives and negatives on the data sheet.

*As an alternative to transfering with a wire loop, sterilized hardwood applicator sticks (sterilized in dry heat, 1-1/2 hours, 170°C, stored in glass tubes or syringe-sterilization bags) may be used. Each stick is used once and then discarded into a disinfectant-filled discard container.

III. Fecal Coliform (MPN)

A. Preparation of medium

1. Weigh out 37.0g of Bacto E.C. medium and add to one liter of cold distilled water.

2. Dissolve the ingredients and dispense into test tubes in ten ml amounts.

3. Insert a Durham gas vial into each tube. (In the inverted position.) Use Kaput closures for these tubes.

4. Sterilize in the autoclave for 12 minutes at 12 lbs. pressure. Final reaction of the medium is pH 6.9.

B. Procedure

*1. Using a 24 gauge wire loop, with a loop of 3 mm in diameter, transfer one loopful of the positive, lauryl tryptose broth into a tube of E.C. medium.

2. The inoculated tube must be put into a 44.5 $\pm$ 0.2°C water bath not later than 20 minutes after initial inoculation.

3. The formation of any amount of gas in the vial at the end of 24 hours constitutes a positive test. At the end of 24 hours the positive and negative results are entered on the data sheet. Readings after 24 hours are invalid.

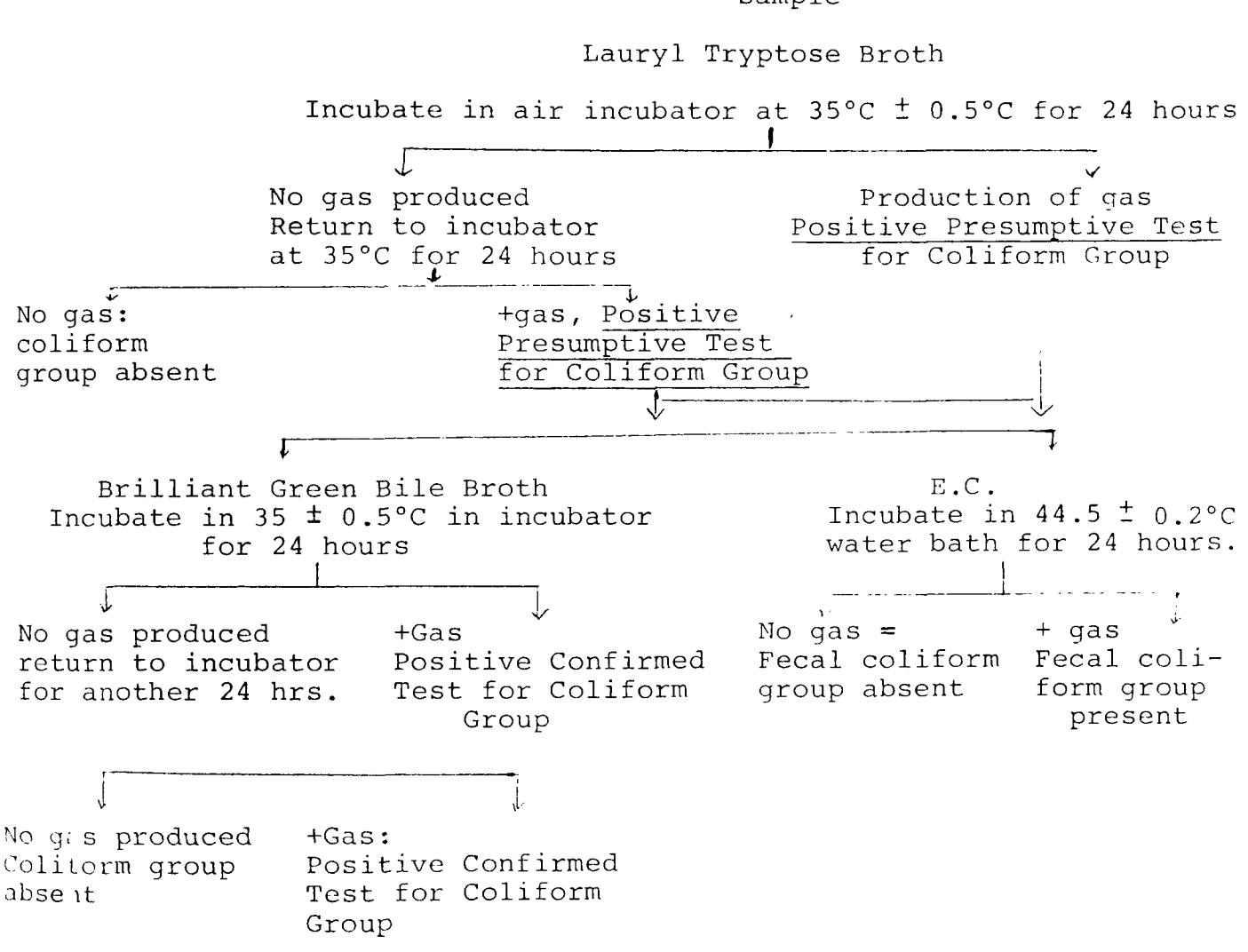
IV. Computing of MPN

1. The number of positive findings of coliform group organisms (Presumptive, Confirmed and Fecal) resulting from multiple-portion, decimal dilution inoculations should be computed and recorded in terms of the "most probable number" (MPN).

2. See MPN and 95% Confidence Limits for Various Combination of Positive Results in Standard Methods, 13th Edition, 1971.

*The single-use, sterile, hardwood applicators may be used as an alternative to transferring with a wire loop.

V. Schematic Diagram Illustrating Steps in MPN Procedures.



THE MOST PROBABLE NUMBER (MPN) TEST FOR THE DETECTION AND ENUMERATION OF FECAL STREPTOCOCCI ORGANISMS

I. Presumptive Test

A. Preparation of medium

1. For 1 ml of sample inoculation: weigh out 34.7 grams Azide Dextrose Broth and add to one liter (1000 ml) of distilled water (for 10 ml of sample inoculation prepare double-strength medium).

2. Dissolve ingredients and dispense 10 ml of mixture into test tubes for 1 ml sample inoculation. Dispense 10 ml of double strength medium into large (18 x 150 mm) test tube for a 10 ml sample inoculum.

3. Place plastic or metal caps on tubes.

4. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121°C). Final reaction of medium is pH 7.2 25°C.

B. Procedure

1. The five-tube, multiple fermentation technique will be used.

2. Inoculate a series of five tubes in <u>each</u> dilution. In all such analyses, at least 3 dilutions must be used (EPA, Region IX routinely uses 5 dilutions).

3. The dilutions of the water sample used for inoculating the fermentation tubes will vary with the type of water being analyzed. Decimal multiples and decimal dilutions of one ml are used.

4. Incubate the inoculated tubes at 35.0 ± 0.5°C. Examine each tube for the presence of turbidity at end of 24 ± two hours. If no definite turbidity is present, reincubate and read again at end of 48 ± three hours.

II. Confirmed Test

A. Preparation of Medium

1. Weigh out 35.8 grams EVA Broth (Ethyl Violet Azide) and add to 1 liter (1000 ml) distilled water.

2. Dissolve and dispense 10 ml portions into test tubes.

3. Sterilize in autoclave for 15 minutes at 15 pounds pressure (121°C). Final reaction of medium pH 7.0 at 25°C.

B. Procedure

1. Transfer 3 loopfuls of growth or use a wooden applicator to transfer growth from each azide dextrose broth tube to a tube containing 10 ml ethyl violet azide broth.

2. Do not discard positive tubes (presumptive). Hold in the incubator.

3. Incubate the inoculated tubes for 24 hours at 35 ± 0.5°C. The presence of fecal streptococci is indicated by formation of a purple button at the bottom of the tube or by a very dense turbidity.

4. Record all positive tubes. Discard those tubes.

5. If no growth (purple button or heavy tubidity) appears in ethyl violet azide in 24 hours, reinoculate the tubes with an additional 3 loopfuls (or use wooden applicator) from the original positive azide broth cultures and reincubate for another 24 hours.

6. Record results.

C. Computing and Recording MPN

Same as with computation of total and fecal coliform organisms.

STANDARD METHODS, APHA, 13th EDITION
407 D. ESTIMATION OF BACTERIAL DENSITY

1. Precision of Fermentation Tube Test

It is desirable to bear in mind that unless a large number of portions of sample are examined, the precision of the fermentation tube test is rather low. For example, even when the sample contains 1 coliform organism per milliliter, about 37% of 1-ml tubes may be expected to yield negative results because of irregular distribution of the bacteria in the sample. When five tubes, each with 1 ml of sample are employed under these conditions, a completely negative result may be expected less than 1% of the time.

Even when five fermentation tubes are employed, the precision of the results obtained is not of a high order. Consequently, great caution must be exercised when interpreting, in terms of sanitary significance, the coliform results obtained from the use of a few tubes with each dilution of sample, especially when the number of samples from a given sampling point is limited.

2. Computing and Recording of MPN

The number of positive findings of coliform group organisms (either presumptive, confirmed or completed) resulting from multiple-portion decimal-dilution plantings should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN). The MPN, for a variety of planting series and results, is given in Tables 407(1) through (6).* Included in these tables are the 95% confidence limits for each MPN value determined.

The quantities indicated at the heads of the columns relate more specifically to finished waters. The values may be used in computing the MPN in larger or smaller portion plantings in the following manner: If, instead of portions of 10, 1.0 and 0.1 ml, a combination of portions of 100, 10 and 1 ml is used, the MPN is recorded as 0.1 times the value given in the applicable table.

*Since Region IX routinely uses the five tube MPN procedure, only Table 407(1), and 407(2) from Standard Methods 13th Ed. are included in this manual.

If, on the other hand, a combination of corresponding portions at 1.0, 0.1 and 0.01 ml is planted, record 10 times the value shown in the table; if a combination of portions of 0.1, 0.01 and 0.001 ml is planted, record 100 times the value shown in the table; and so on for other combinations.

When more than three dilutions are employed in a decimal series of dilutions, the results from only three of these are used in computing the MPN. To select the three dilutions to be employed in determining the MPN index, taking the system of five tubes of each dilution as an example, the highest dilution which gives positive results in all five portions tested (no lower dilution giving any negative results) and the next succeeding higher dilutions should be chosen. The results at these three volumes should then be used in computing the MPN index. In the examples given below, the significant dilution results are shown in boldface. The number in the numerator represents positive tubes; that in the denominator, the total tubes planted; the combination of positives simply represents the total number of positive tubes per dilution:

Example	1 ml	0.1 ml	0.01 ml	0.001 ml	Combination of positives
(a)	5/5	5/5	2/5	0/5	5-2-0
(b)	5/5	4/5	2/5	0/5	5-4-2
(c)	0/5	1/5	0/5	0/5	0-1-0

In c, the first three dilutions should be taken, so as to throw the positive result in the middle dilution.

When a case such as shown below in line d arises, where a positive occurs in a dilution higher than the three chosen according to the rule, it should be incorporated in the result for the highest chosen dilution, as in e:

Example	1 ml	0.1ml	0.01ml	0.001 ml	Combination of positives
(d)	5/5	3/5	1/5	1/5	5-3-2
(e)	5/5	3/5	2/5	0/5	

When it is desired to summarize with a single MPN value the results from a series of samples, the geometric mean, the arithmetic mean, or the median may be used.

TABLE 407(1): MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction out of 5 of 10 ml Each	MPN Index per 100 ml	95% Confidence Limits	
		Lower	Upper
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.	3.3	52.9
5	>16.	8.0	Infinite

Formula* for MPN Calculation:

$$\text{MPN/100 ml} = \frac{\text{Number Positive Tubes} \times 100}{\sqrt{(\text{ml sample in negative tubes}) \times (\text{ml sample in all tubes})^2}}$$

*From Thomas, H.A. Jr. 1942. Bacterial densities from fermentation tube tests. JAWWA 34:572.

TABLE 407(2): MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS, FIVE 1-ML PORTIONS AND FIVE 0.1-ML PORTION ARE USED

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each		Lower	Upper
0	0	0	< 2		
0	0	1	2	< 0.5	7
0	1	0	2	< 0.5	7
0	2	0	4	< 0.5	11
1	0	0	2	< 0.5	7
1	0	1	4	< 0.5	11
1	1	0	4	< 0.5	11
1	1	1	6	< 0.5	15
1	2	0	6	< 0.5	15
2	0	0	5	< 0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67

ntinued on page 22

(Cont'd. from page 21)

TABLE 407(2): MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS, FIVE 1-ML PORTIONS AND FIVE 0.1-ML PORTION ARE USED

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml	95% Confidence Limits	
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each		Lower	Upper
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1,000
5	5	0	240	68	750
5	5	1	350	120	1,000
5	5	2	540	180	1,400
5	5	3	920	300	3,200
5	5	4	1600	640	5,800
5	5	5	>2400		

GRAMS STAIN

1. Using a clean slide, make a thin smear of the culture on the slide.

2. Air dry

3. Heat fix by passing slide briefly through flame.

4. Add gentian violet dye - let stand one minute. Rinse with tap water. Rinse with Gram's iodine.

5. Add Gram's iodine - let stand one minute. Drain slide.

6. Add decolorizer - let stand 10-15 seconds. Rinse with water. Rinse with Safranin.

7. Add Safranin dye to counterstain - one minute. Rinse with tap water. Blot dry with paper towel.

View slide under microscope.

Gram-positive organisms appear blue-violet; gram-negative bacteria retain only the counterstain, appearing red when Safranin is counterstain.

IMViC PROCEDURES
(Standard Methods, 13th Ed.)

Be sure to use a pure culture for all IMViC inoculations.

Indole production from tryptophane broth

1. Prepare Medium:

   Add 10.0 g. tryptone (or trypticase) to 1 liter distilled water. Distribute in 5 ml portions into screw-capped test tubes. Autoclave at 120°C for 15 minutes.

2. Prepare reagent:

   Dissolve 5 g. paradimethylaminobenzaldehyde in 75 ml isoamyl (or normal amyl) alcohol, ACS grade. Add 25 ml concentrated hydrochloric acid (perform this operation under a hood if possible or else in a room with good ventilation). The reagent should turn yellow. Final pH should be less than 6.0.

3. Procedure:

   Inoculate 5 ml medium. Incubate at 35 ± 0.5°C for 24 ± 2 hours. Add 0.2-0.3 ml reagent and shake tube. Let stand for 10 minutes.

4. Results:

   Dark red color in surface layer = (+) for indole. Yellow (Color of reagent) color in surface layer = (-) for indole. Orange color in surface layer = (±) reaction.

Methyl Red Test

1. Prepare Medium:

   Add 17 g. MR-VP Broth Base (or equivalent) to 1 liter distilled water. Heat slightly to dissolve. Distribute 10-ml portions into screw-capped test tubes. Autoclave at 121°C for 12-15 minutes.

2. Prepare reagent:

   Dissolve 0.1 g. methyl red dye in 300 ml 95% ethyl alcohol. Dilute to 500 ml total volume with distilled water.

3. Procedure:

   Inoculate 10-ml medium. Incubate at 35°C for 5 days.

After incubation, pipette 5 ml culture into a clean test tube. Add 5 drops (about 0.25 ml) methyl red reagent.

4. Results:

Distinct red color = (+) methyl red. Distinct yellow color = (-) methyl red. Mixed shade = questionable results.

Voges-Proskauer Test

1. Prepare medium:

The same tube of MR-VP broth inoculated for the methyl red tests may be used for the Voges-Proskauer test as well.

2. Prepare reagents:

Naphthol solution: Dissolve 5 g. purified naphthol (melting point 92.5 or higher) in 100 ml absolute ethyl alcohol. Solution should be prepared fresh each day.

Potassium Hydroxide solution: Dissolved 40 g. KOH in 100 ml distilled water.

3. Procedure:

Inoculate 5 ml medium. Incubate at 35 ± 0.5°C for 48 hours. Pipette 1 ml culture into a clean test tube. Add to this 0.6 ml naphthol solution and 0.2 ml KOH solution.

4. Results:

Pink to crimson color develops in 2-4 hours = (+) for V-P.

Sodium Citrate Test

1. Prepare medium:

Add 24.2 g. Simmons Citrate Agar to 1 liter distilled water. Mix thoroughly. Heat with frequent agitation until medium boils for one minute. Distribute into screw-capped test tubes. Autoclave at ~~120~~ 121°C for 15 minutes. Cool tubes in slanted position.

2. Procedure:

Inoculate the medium with a straight needle, using both a stab and a streak. Incubate 48 hours at 35 ± 0.5°C.

3. Results:

Growth on the medium with a blue color (usually) = (+) for citrate utilization. No growth = (-) test.

PROCEDURE FOR SALMONELLA ISOLATION

(for shellfish or sediment modifications see note following Serological Grouping)

Water Samples, Filtration

1. Set up filter system. Millipore assembly may be used. Substitute filter pad for actual filter grid. On top of pad (with funnel in place) add one inch of diatomaceous earth (Celite or equivalent). Add sterile buffered water to saturate Celite column.
2. Filter one liter sample.
3. Add filter pad plus diatomaceous earth to enrichment broth. Repeat filtration procedure for each flask of enrichment broth (a total of six filtrations for two broths and three temperatures).

Enrichment

1. Two enrichment broths (tetrathionate and selenite) should be used at each of three different temperatures (37°, 41.5°, 43°C). In case of limited laboratory capability the 43°C incubation may be omitted.
2. Incubate 18-24 hours at appropriate temperature.

Isolation

1. From the enrichment flask make streak plates and plates for impression smears onto Brilliant Green and XLD agars (reincubate enrichment broth). Incubate plates at same temperature as for flasks.
2. Time of incubation for streak plates is indicated in Table 1. Impression smears should be made on slides after 2-4 hours incubation.
3. Smears should be stained and examined as indicated in fluorescent-antibody staining directions. If no fluorescing cells (3+, 4+) are found, sample may be said to contain no Salmonellae. If fluorescing cells are present, continue isolation procedure (steps 4-5); streaking from enrichment broths may be repeated after 2 and 3 days incubation (or up to the point at which no Salmonella-like colonies are recovered).
4. From each medium select isolated colonies of Salmonella-like appearance (consult Table 1) and restreak until pure cultures are obtained. Incubate at 37°C.
5. Inoculate isolates into differential media in order indicated.

Differential Tests

1. Using a single colony, inoculate 1/2 into a Triple Sugar Iron slant and the other 1/2 into urea agar. Incubate at 37°C (times are indicated in Table 1).
2. Those isolates with Salmonella-like reactions in both TSI and urea should be inoculated into the following: Brilliant Green

and XLD agar plates (streak); SIM (stab); Lysine Decarboxylase; Nutrient Agar. Use material from the TSI slant for these inoculations. Incubate at 37°C for times indicated in Table 1.

Serological Grouping

1. Isolates displaying a Salmonella-like pattern in differential media may be grouped according to their somatic antigens. This agglutination reaction may be lost when cultures are not freshly isolated.
2. To prime the isolate for the agglutination test, inoculate it into a Brain Heart Infusion slant (or broth). Incubate 24 hours at 37°C.
3. Repeat step 2 at least once or twice, ending up with a fresh BHI slant.
4. Perform slide agglutination tests as indicated in directions accompanying Difco Salmonella O Antisera. Perform the agglutination test for each of the sets of antisera (Sets A-1, A, B, C, D, E, F, G).
5. Final typing for O and H antigens may be done at a convenient typing center (California State Public Health, Berkeley).

MODIFICATIONS FOR SEDIMENT OR SHELLFISH SAMPLES

Sediment

1. Omit filtration procedure.
2. Inoculate sediment directly into enrichment broth. For oily sample, use 1 gm. For other material, use 10 gm.
3. Proceed as directed through outline.

Shellfish

1. Omit filtration procedure.
2. As directed in Recommended Procedures for the Examination of Sea Water and Shellfish (1970), weigh 100 gm (approximately) of shellfish meat and liquor and add an equal weight of buffered dilution water. Grind in a blender about 2 minutes. Pipet 20 ml into each enrichment flask.
3. Proceed as directed through outline.

Table 1: Incubation Time and Colonial Appearance for Various Organisms in Selected Media

Medium	Incubation (Hours)	Colonial Appearance			
		Salmonella	Shigella	Proteus	Coliforms
Brilliant Green	48	pinkish with red background	-	green	green
XLD	24	red with black ctr.	red	yellow	yellow
Lysine Decarboxylase	24	+(purple)	-(yellow)	-	+,-
Indole	24	-	+,-	+,-	+,-
Motility	24	+	-	+	+,-
Urease	24	-	-	+	-
TSI-slant	18-48	Al	Al	A	A
-butt		AG	A	AG	AG
-H_2S		+,-	-	+,-	+,-

A = acid production (indicator turns yellow)
Al = alkaline reaction (indicator turns red)
G = gas formation (bubbles appear in agar)

Rapid Procedure - In addition to and supplement of Table 1:

Omit Indole, Motility, Urease, TSI, lysine decarboxylase.

Inoculate Improved Enterotube. Instructions in use of Enterotube accompany package.

The Enterotube is prepared by Roche Diagnostics, Division of Hoffmann - La Roche, Inc., Nutley, New Jersey, 07110.

Table II shows parameters and biochemical reactions for Salmonelleae (Salmonella, Arizona, Citrobacter).

TABLE II SALMONELLEAE
Parameters and Biochemical Reactions

TEST OR SUBSTRATE	SALMONELLEAE		
	Salmonella	Arizona	Citrobacter
Indol	-	-	-
Methyl Red	+	+	+
Voges - Proskauer	-	-	-
Simmons' Citrate	d	+	+
Hydrogen Sulfide (TSI)	+	+	+ or -
Urease	-	-	d^{w}
KCN	-	-	+
Motility	+	+	+
Gelatin (22°C)	-	(+)	-
Lysine Decarboxylase	+	+	-
Arginine Dihydrolase	(+) or +	+ or (+)	d
Ornithine Decarboxylase	+	+	d
Phenylalanine Deaminase	-	-	-
Malonate	-	+	d
Gas From Glucose	+	+	+
Lactose	-	d	d
Sucrose	-	-	d
Mannitol	+	+	+
Dulcitol	$d^{(1)}$	-	d
Salicin	-	-	d

(Continued next page)

TABLE II SALMONELLEAE
Parameters and Biochemical Reactions

TEST OR SUBSTRATE	SALMONELLEAE		
	Salmonella	Arizona	Citrobacter
Adonitol	-	-	-
Inositol	d	-	-
Sorbitol	+	+	+
Arabinose	+(1)	+	+
Raffinose	-	-	d
Rhamnose	+	+	+

(1) S. typhi, S. cholerae-suis, S. enteritidis bioser. Paratyphi A and Pullorum, and a few others ordinarily do not ferment dulcitol promptly. S. cholerae-suis does not ferment arabinose.

+, 90 percent or more positive in 1 or 2 days. -, 90 percent or more negative. d, different biochemical types [+,(+),-]. (+), delayed positive. + or -, majority of cultures positive. - or +, majority negative. w, weakly positive reaction.

Compiled by Difco Laboratories, Detroit, Michigan

Standard Method used
by
Region IX Laboratory

FA - SALMONELLA SCREENING

1. From colony or agar slant, make light saline suspension. (Use fresh agar slant culture and suspend in a small amount of solution made by mixing 0.85 g NaCl to 100ml distilled water.
2. Prepare smears of this suspension on clear glass FA slides (1.0 to 1.1 mm thick).
3. Air dry the smears - then fix for 2 minutes in Kirkpatrick's Fixative. Rinse briefly in 95% ethanol. Allow to dry. Do not blot.
4. Cover the fixed smears with one drop of Salmonella polyvalent OH conjugate. (use 1:8 dilution of the conjugate)

*5. Place slides in a moist chamber to prevent evaporation of the staining reagent. After 30 minutes, wash away excess reagent by dipping slide into buffered saline (pH 7.5 - 8.0).

6. Place slide in second bath of buffered saline for 10 minutes.
7. Remove slides, rinse in distilled H_2O) and allow to drain dry.
8. Place a small drop of mounting fluid on the smear and cover with a No. 1 coverslip. Examine under fluorescence scope, using UG-1 (2 mm) primary filter and GG-9 (1 mm) ocular filter. A combination of BG-12 (3mm) and OG-1 (1 mm) will also give satisfactory results.

Kirkpatrick's Fixative

60 ml absolute ethanol

30 ml chloroform

10 ml formalin

*Buffered Saline: Bacto-FA Buffer Dried, prepared by Difco Laboratories is recommended. Instructions for preparation accompany the package.

F. Brezenski
Region II

TECHNIQUES USED BY REGION IX FOR SEROLOGICAL GROUPING OF SALMONELLA

1. After completion of Differential Test, choose an isolated colony from Brilliant Green Agar and/or XLD plates which were streaked from Triple Sugar Iron slant (to obtain well isolated colonies it may be necessary to re-streak several times).

2. From Brilliant Green Agar and/or XLD plates, pick well isolated Salmonella-like colony. Inoculate it into Brain Heart Infusion broth. Incubate 24 hours at 37°C.

3. Repeat priming of isolate by transferring a loopful of Brain Heart Infusion (BHI) broth culture into a fresh tube of BHI broth. After 3-4 transfers, inoculate onto BHI slant. Make two slants (always keep one for stock).

4. Perform slide agglutination test (see also page 27 Salmonella Procedures, Serological Grouping, Step 4).

   a. Prepare a dense suspension of organism from fresh 18-hour BHI slant in 0.5 ml of 0.85% sodium chloride solution. Suspension should be homogeneous and at least as concentrated as that of Bacto McFarland Barium Sulfate Standard #10 (which corresponds to $3x10^9$ cells/ml).

   b. Using alcohol-cleaned slide mark slide into 4 sections 1 cm square. Using wax pencil, mark heavily (form continuous lines) to avoid spilling from one section to another.

   c. Place one drop (use capillary pipet with rubber bulb) of 0.05 ml of the Bacto-Salmonella O Antiserum Poly within one square.

   d. To the square next to antiserum, place one drop of 0.85% sodium chloride solution (This serves as negative control).

   e. Using a clean inoculating loop, transfer a loopful (0.05 ml) of bacterial suspension in 0.85% sodium chloride prepared in step a and gently mix to emulsify thoroughly.

   f. Transfer another loopful of bacterial suspension to section containing antiserum.

   g. Gently rock the slide 1-2 minutes watching for agglutination. (Using a small inverted fluorescent lamp aids in detecting agglutination process.)

Positive agglutination is rapid. Delayed agglutination (over 2 minutes) or partial agglutination should be considered negative.

5. If culture reacts with Bacto-Salmonella O Antiserum Poly (step g) but does not react with the specific Salmonella O Antiserum groups, it should be checked with Bacto-Salmonella Vi Antiserum by same method as described above. If the culture does not agglutinate with Salmonella Vi antiserum, the culture may be regarded as not of the Salmonella genus.

   If the culture does react with the Vi antiserum, proceed as follows:

   a. Heat the culture suspension in a boiling water bath for 10 minutes, cool.

   b. After cooling the heated culture should be re-tested with the desired individual Salmonella O antiserum groups and the Salmonella Vi antiserum.

6. If the organism does not react with the Vi antiserum after heating, it is ready to be confirmed by a Public Health Laboratory or typing center.

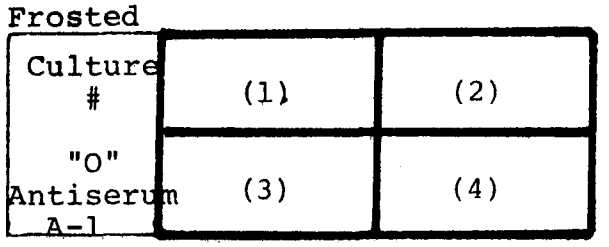
   a. Send a pure culture slant.

   b. List all parameters which were performed and results obtained. Send along with culture slant.

   c. It is preferable to deliver culture to Public Health Laboratory; however, if this cannot be done, the culture (in a screw-capped tube) should be carefully packed inside a metal screw cap tube and then into a mailing tube properly labelled and sent as registered mail.

Attachment: Diagram of slide agglutination

Diagram of slide agglutination



(Example)

Wax pencil enclosure

Slide Section	Drop to contain
#1	Antiserum alone
#2	Antiserum + 0.85% NaCl
#3	Bacterial Suspension in 0.85% NaCl
#4	Bacterial Suspension in 0.85% NaCl + Antiserum

ENVIRONMENTAL PROTECTION AGENCY
Water Quality Office
Water Hygiene Division

Bacteriological Survey for
Water Laboratories

Indicating conformity with the 13th edition of Standard Methods for the Examination of Water and Wastewater (1971).

Survey By	X = Deviation U = Undetermined O = Not Used	
Laboratory	Location	Date

Sampling and Monitoring Response

1. Location and Frequency
   - Representative points on system. . . . . ____
   - Frequency of sampling adequate. . . . . ____

2. Collection Procedure
   - Faucets with aerators should not be used. . . . . ____
   - Flush tap 1 min. prior to sampling . . . . . ____
   - Pump well 1 min. to waste prior to sampling . . . . . ____
   - River, stream, lake, or reservoir sampled at least 6 inches below surface and toward current. . . . . ____
   - Minimum sample not less than 100 ml . . . . . ____
   - Ample air space in bottle for mixing. . . . . ____
   - Promptly identify sample legibly and indelibly . . . . . ____

3. Sample Bottles
   - Wide mouth, glass or plastic bottles of _____ capacity. . . . . ____
   - Sample bottles capable of sterilization and rinse . . . . . ____
   - Closure:
     a. Glass stoppered bottles protected with metal foil, rubberized cloth or kraft type paper . . . . . ____
     b. Metal or plastic screw cap with leakproof liner . . . . . ____
   - Sodium thiosulfate added for dechlorination. . . . . ____
     Concentration 100 mg/l added before sterilization . . . . . ____
   - Chelation agent for stream samples (optional). . . . . ____
     Concentration 372 mg/l added before sterilization . . . . . ____

4. Transportation and Storage
   - Complete and accurate data accompanies sample . . . . . ____
   - Transit time for potable water samples should not exceed 48 hrs, preferably within 30 hrs . . . . . ____
   - Transit time for source waters, reservoirs, and natural bathing waters should not exceed 6 hrs . . . . . ____
   - All samples examined within 2 hours of arrival . . . . . ____

Laboratory	Location	Date

4. Transportation and Storage (Continued)

Sample refrigeration mandatory on stream samples, optional on potable water samples. . . . . ____

5. Record of Laboratory Examination

Results assembled and available for inspection . . . . . ____

Number of Tests per year

MPN Test - Type of sample _____

Confirmed (+)_____ (-)_____ (Total)_____

Completed (+)_____ (-)_____ (Total)_____

MF Test - Type of sample _____

Direct Count (+)_____ (-)_____ (Total)_____

Verified Count (+)_____ (-)_____ (Total)_____

Data processed rapidly through laboratory and engineering sections . . ____

Unsatisfactory sample defined as 3 or more positive tubes per MPN test or 5 or more colonies per 100 ml in MF test . . . . . ____

High priority placed on alerting operator to unsatisfactory potable water results . . . . . ____

Prompt resampling for unsatisfactory samples . . . . . ____

6. Laboratory Evaluation Service

State program to evaluate all laboratories which examine potable water supplies. . . . . ____

Frequency of surveys on a _____year basis. . . . . ____

State survey officer (Name) _____ . . . . ____

Status of laboratory evaluation service. . . . . ____

Total _____labs known to examine water

_____approved laboratories

_____provisional laboratories

Laboratory Apparatus

7. Incubator

Manufacturer _____ Model _____

Sufficient size for daily work load . . . . . ____

Maintain uniform temperature in all parts (± 0.5°C). . . . . ____

Accurate thermometer with bulb immersed in liquid on top and bottom shelves. . . . . ____

Daily record of temperature or use of recording thermometer sensitive to 0.5°C change . . . . . ____

Incubator not subject to excessive room temperature variations beyond a range of 50 - 80°F . . . . . ____

Laboratory	Location	Date

8. Incubator Room (Optional) Manufacturer _____

Well insulated, equipped with properly distributed heating and humidifying units for optimum environmental control. . . . . _____

Shelf areas used for incubation must conform to 35°C ± 0.5° temperature requirement. . . . . _____

Accurate thermometers with bulb immersed in liquid. . . . . _____

Daily record of temperature at selected areas or use recording thermometer sensitive to 0.5°C changes . . . . . _____

9. Water Bath

Manufacturer _____ Model _____

Sufficient size for fecal coliform tests . . . . . _____

Maintain uniform temperature 44.5°C ± 0.2°C. . . . . _____

Accurate thermometer immersed in water bath . . . . . _____

Daily record of temperature or use of recording thermometer sensitive to 0.2°C changes . . . . . _____

10. Hot Air Sterilizing Oven

Manufacturer _____ Model _____

Size sufficient to prevent crowding of interior . . . . . _____

Constructed to insure a stable sterilizing temperature . . . . . _____

Equipped with accurate thermometer in range of 160-180°C or with recording thermometer . . . . . _____

11. Autoclave

Manufacturer _____ Model _____

Size sufficient to prevent crowding of interior . . . . . _____

Constructed to provide uniform temperature up to and including 121°C . . . . . _____

Equipped with accurate thermometer with bulb properly located to register minimal temperature within chamber . . . . . _____

Pressure gage and operational safety valve . . . . . _____

Steam source from saturated steam line, or from gas or electrically heated steam generator . . . . . _____

Reach sterilization temperature in 30 min. . . . . _____

Pressure cooker may be used only if provided with a pressure gage and thermometer with bulb 1 in. above water level . . . . . _____

12. Thermometers

Accuracy checked with thermometer certified by National Bureau of Standards or one of equivalent accuracy. . . . . _____

Liquid column free of discontinuous sections and graduation marks legible . . . . . _____

Laboratory	Location	Date

13. pH Meter

Manufacturer_____ Model_____

Electronic pH meter accurate to 0.1 pH units. . . . . ____

14. Balance

Balance with 2 g sensitivity at 150 g load used for general media preparations, Type_____ . . . . . ____

Analytical balance with 1 mg sensitivity at 10 g load used for weighing quantities less than 2 g, Type_____ . . . ____

Appropriate weights of good quality for each balance . . . . . ____

15. Microscope and Lamp

Preferably binocular wide field, 10 to 15 diameters magnification for MF colony counts, Type_____ . . . . . ____

Fluorescent light source for sheen discernment. . . . . ____

16. Colony Count

Quebec colony counter, dark-field model preferred for standard plate counts . . . . . ____

17. Inoculating Equipment

Wire loop of 22 or 24 gauge chromel, nichrome, or platinum iridium, sterilized by flame . . . . . ____

Single-service transfer loops of aluminum or stainless steel, presterilized by dry heat or steam. . . . . ____

Disposable single service hardwood applicators, presterilized by dry heat only. . . . . ____

18. Membrane Filtration Units

Manufacturer_____ Type_____

Leak proof during filtration. . . . . ____

Metal plating not worn to expose base metal . . . . . ____

19. Membrane filters

Manufacturer_____ Type_____

Full bacterial retention, satisfactory filtration speed . . . . . ____

Stable in use, glycerin free. . . . . ____

Grid marked with non-toxic ink . . . . . ____

Presterilized or autoclaved 121°C for 10 min. . . . . ____

20. Absorbent Pads

Manufacturer_____ Type_____

Filter paper free from growth inhibitory substances. . . . . ____

Thickness uniform to permit 1.8 - 2.2 ml medium absorption . . . . ____

Presterilized or autoclaved with membrane filters . . . . . ____

Laboratory	Location	Date

21. Forceps

Preferably round tip without corrugations. . . . . ____
Forceps are alcohol flamed for use in MF procedure. . . . . ____

Glassware, Metal Utensils and Plastic Items

22. Media Preparation Utensils

Borosilicate glass . . . . . ____
Stainless steel. . . . . ____
Utensils clean and free from foreign residues or dried medium. . . . . ____

23. Pipets

Brand _____ Type _____
Calibration error not exceeding 2.5%. . . . . ____
Tips unbroken, graduation distinctly marked . . . . . ____
Deliver accurately and quickly. . . . . ____
Mouth end plugged with cotton (optional) . . . . . ____

24. Pipet Containers

Box, aluminum or stainless steel . . . . . ____
Paper wrapping of good quality sulfite paper (optional) . . . . . ____

25. Petri Dishes

Brand _____ Type _____
Use 100 mm x 15 mm dishes for pour plates . . . . . ____
Use 60 mm x 15 mm dishes for MF cultures . . . . . ____
Clear, flat bottom, free from bubbles and scratches. . . . . ____
Plastic dishes may be reused if sterilized in 70% ethanol for 30 min. or by ultraviolet radiation . . . . . ____

26. Petri Dish Containers

Aluminum or stainless steel cans with covers, coarsely woven wire baskets, char-resistant paper sacks or wrappings . . . . . ____

27. Culture Tubes

Size sufficient for total volume of medium and sample portions . . . . ____
Borosilicate glass or other corrosive resistant glass . . . . . ____

28. Dilution Bottles or Tubes

Borosilicate or other corrosive resistant glass . . . . . ____
Screw cap with leak-proof liner free from toxic substances on sterilization . . . . . ____
Graduation level indelibly marked on side of bottle or tube . . . . . ____

Laboratory	Location	Date

Materials and Media Preparation

29. Cleaning Glassware

Dishwasher Manufacturer _____ Model _____

Thoroughly washed in detergent at 160° F, cycle time _____ . . ____

Rinse in clean water at 180° F, cycle time _____ . . . . . ____

Final rinse in distilled water, cycle time _____ . . . . . ____

Detergent brand _____

Washing procedure leaves no toxic residue . . . . . ____

Glassware free from acidity or alkalinity . . . . . ____

30. Sterilization of Materials

Dry heat sterilization (1 hr at 170° C)

Glassware not in metal containers . . . . . ____

Dry heat sterilization (2 hrs at 170° C)

Glassware in metal containers. . . . . ____

Glass sample bottles . . . . . ____

Autoclaving at 121° C for 15 min . . . . . ____

Plastic sample bottles . . . . . ____

Dilution water blanks . . . . . ____

31. Laboratory Water Quality

Still manufacturer _____ Construction Material _____

Demineralizer with _____ recharge frequency

Protected storage tank . . . . . ____

Supply adequate for all laboratory needs. . . . . ____

Free from traces of dissolved metals or chlorine . . . . . ____

Free from bactericidal compounds as measured by bacteriological suitability test . . . . . ____

Bacteriological quality of water measured once each year by suitability test or sooner if necessary . . . . . ____

32. Buffered Dilution Water

Stock phosphate buffer solution pH 7.2 . . . . . ____

Prepare fresh stock buffer when turbidity appears . . . . . ____

Stock buffer autoclaved and stored at 5 - 10° C . . . . . ____

1.25 ml stock buffer per 1 liter distilled water. . . . . ____

Dispense to give 99 ± 2 ml or 9 ± 0.2 ml after autoclaving. . . . . ____

33. pH Measurements

Calibrate pH meter against appropriate standard buffer prior to use . . ____

Standard buffer brand _____ pH _____

Check the pH of each sterile medium batch or at least one batch from each new medium lot number. . . . . ____

Laboratory	Location	Date

33. pH Measurements (Continued)
Maintain a pH record of each sterile medium batch, the date and lot number. . . . . ____

34. Sterilization of Media
Carbohydrate medium sterilized 121° C for 12 min . . . . . ____
All other media autoclaved 121° C for 15 min . . . . . ____
Tubes packed loosely in baskets for uniform heating and cooling . . . . ____
Timing starts when autoclave reaches 121° C . . . . . ____
Total exposure of carbohydrate media to heat not over 45 min . . . . . ____
Media removed and cooled as soon as possible after sterilization . . . ____

35. Storage
Dehydrated media bottles kept tightly closed and stored at less than 30° C. . . . . ____
Dehydrated media not used if discolored or caked . . . . . ____
Sterile culture media stored in clean area free from contamination and excessive evaporation . . . . . ____
Sterile batches used in less than 1 week. . . . . ____
All media protected from sunlight . . . . . ____
If media is stored at low temperatures, it must be incubated overnight and any tubes with air bubbles discarded . . . . . ____

Culture Media - Specifications

36. Lactose Broth
Manufacturer _____ Lot No. _____
Single strength composition 13 g per liter distilled water . . . . . ____
Single strength pH 6.9 ± 0.1, double strength pH 6.7 ± 0.1 . . . . . ____
Not less than 10 ml medium per tube . . . . . ____
Composition of medium after 10 ml sample is added must contain 0.013 g per ml dry ingredients . . . . . ____

37. Lauryl Tryptose Broth
Manufacturer _____ Lot No. _____
Single strength composition 35.6 g per liter distilled water . . . . . ____
Single strength pH 6.8 ± 0.1, double strength pH 6.7 ± 0.1 . . . . . ____
Not less than 10 ml medium per tube . . . . . ____
Composition of medium after 10 ml sample is added must contain 0.0356 g per ml of dry ingredients . . . . . ____

38. Brilliant Green Lactose Bile Broth
Manufacturer _____ Lot No. _____

Laboratory	Location	Date

38. Brilliant Green Lactose Bile Broth (Continued)
    - Correct composition, sterility and pH 7.2. . . . . ____
    - Not less than 10 ml medium per tube . . . . . ____

39. Eosin Methylene Blue Agar
    - Manufacturer _____ Lot No. _____
    - Medium contains no sucrose, Cat. No. _____
    - Correct composition, sterility and pH 7.1. . . . . ____

40. Plate Count Agar (Tryptose Glucose Yeast Agar)
    - Manufacturer _____ Lot No. _____
    - Correct composition, sterility and pH 7.0 ± 0.1 . . . . . ____
    - Free from precipitate. . . . . ____
    - Sterile medium not remelted a second time after sterilization. . . . . ____

41. EC Medium
    - Manufacturer _____ Lot No. _____
    - Correct composition, sterility and pH 6.9. . . . . ____
    - Not less than 10 ml medium per tube . . . . . ____

42. M-Endo Medium
    - Manufacturer _____ Lot No. _____
    - Correct composition and pH 7.1 - 7.3 . . . . . ____
    - Reconstituted in distilled water containing 2% ethanol. . . . . ____
    - Heat to boiling point, promptly remove and cool . . . . . ____
    - Store in dark at 2 - 10°C . . . . . ____
    - Unused medium discarded after 96 hrs . . . . . ____

43. M-FC Broth
    - Manufacturer _____ Lot No. _____
    - Correct composition and pH 7.4 . . . . . ____
    - Reconstituted in 100 ml distilled water containing 1 ml of a 1% rosolic acid reagent. . . . . ____
    - Stock solution of rosolic acid discarded after 2 weeks or when red color changes to muddy brown . . . . . ____
    - Heat to boiling point, promptly remove and cool . . . . . ____
    - Store in dark at 2 - 10°C . . . . . ____
    - Unused medium discarded after 96 hrs . . . . . ____

44. _____ Broth
    - Manufacturer _____ Lot No. _____
    - Correct composition and pH . . . . . ____

45. _____ Agar
    - Manufacturer _____ Lot No. _____

Laboratory	Location	Date

45. _____ Agar (Continued)

Correct composition and pH. . . . . _____

Multiple Tube Coliform Test

46. Presumptive Procedure

Lactose broth _____ lauryl tryptose broth _____

Shake sample vigorously . . . . . _____

Potable water: 5 standard portions, either 10 or 100 ml. . . . . _____

Stream monitoring: multiple dilutions . . . . . _____

Incubate tubes at 35° ± 0.5°C for 24 ± 2 hr . . . . . _____

Examine for gas _____ any gas bubble positive. . . . . _____

Return negative tubes to incubator. . . . . _____

Examine for gas at 48 ± 3 hr from original incubation . . . . . _____

47. Confirmed Test

Promptly submit all presumptive tubes showing gas production before or at 24 hr and 48 hr periods to Confirmed Test . . . . . _____

a. Brilliant green lactose broth

Gently shake presumptive tube or mix by rotating . . . . . _____

Transfer one loopful of positive broth or one dip of applicator from presumptive tube to brilliant green lactose broth. . . . . _____

Incubate at 35° ± 0.5°C and check at 24 hrs for gas production. . . _____

Reincubate negative tubes for additional 24 hrs and check for gas production . . . . . _____

Calculate MPN or report positive tube results. . . . . _____

b. Endo or eosin methylene blue agar plates adequate streaking to obtain discrete colonies separated by 0.5 cm. . . . . _____

Incubate at 35° ± 0.5°C for 24 ± 2 hr . . . . . _____

Typical nucleated colonies with or without sheen are coliforms . . _____

If atypical unnucleated pink colonies develop, result is doubtful and completed test must be applied. . . . . _____

If no colonies or only colorless colonies appear, the confirmed test is negative. . . . . _____

48. Completed Test

Applied to all potable water samples or a proportion each three months to establish the validity of the confirmed test in determining their sanitary quality. . . . . _____

Applied to positive confirmed tubes or to doubtful colonies on differential medium. . . . . _____

Streak positive confirmed tubes on Endo or EMB plates for colony isolation. . . . . _____

Laboratory	Location	Date

48. Completed Test (Continued)

Choice of selected isolated colony for verification should be one typical or two atypical to lactose or lauryl tryptose broth and to agar slant for Gram stain. . . . . ____
Incubate at 35°C ± 0.5°C for 24 hrs or 48 hrs . . . . . ____
Gram negative rods without spores and gas in lactose tube with 48 hrs in positive Completed Test . . . . . ____

Membrane Filter Coliform Test

49. Application as Standard Test

Use as a standard test for determining potability of water after demonstration by parallel testing that it yields information equal to that from the multiple-tube fermentation procedure . . . . ____

50. MF Procedure

Filter funnel and receptacle sterile at start of series . . . . . ____
Rapid funnel resterilization by UV, flowing steam or boiling water acceptable. . . . . ____
Membrane filter cultures and technician eyes should not be subject to UV radiation leaks . . . . . ____
Filtration volume not less than 50 ml for potable water; multiple dilutions for stream pollution . . . . . ____
Rinse funnel by flushing several 20 - 30 ml portions of sterile buffered water through MF . . . . . ____
Remove filter with sterile forceps . . . . . ____
Roll filter over M-ENDO medium pad or agar so air bubbles will not form . . . . . ____

51. Incubation

In high humidity or in tight fitting culture dishes . . . . . ____
At 35°C ± 0.5°C for 22 - 24 hrs . . . . . ____

52. Counting

All colonies with a metallic yellowish green surface sheen . . . . . ____
If coliforms are found in potable samples, verify by transfers to lactose broth, then to BGB broth for evidence of gas production at 35°C within 48 hr limit. . . . . ____
Calculate direct count in coliform density per 100 ml. . . . . ____

53. Standard MF test with Enrichment

Incubate MF after filtration on pad saturated with lauryl tryptose broth for 1 1/2 - 2 hr at 35°C ± 0.5°C . . . . . ____

Laboratory	Location	Date

53. Standard MF test with Enrichment (Continued)

Transfer MF culture to M-Endo medium for a final 20 - 22 hr incubation at 35°C ± 0.5°C. . . . . ____

Count sheen colonies, verify if necessary, and calculate direct count in coliform density per 100 ml . . . . . ____

Supplementary Bacteriological Methods

54. Standard Plate Count

Plate not more than 1 or less than 0.1 ml (sample or dilution) . . . . . ____

Add 10 ml or more liquefied agar medium at a temperature between 43 - 45°C . . . . . ____

Melted medium stored for no more than 3 hr at 43 - 45°C . . . . . ____

Liquid agar and sample portion thoroughly mixed by gently rotating to spread mixture evenly . . . . . ____

Count only plates with between 30 and 300 colonies, exception being 1 ml sample with less than 30 colonies . . . . . ____

Record only two significant figures and calculate as "standard plate count at 35°C per 1 ml of sample". . . . . ____

55. Fecal Coliform Test

a. Multiple Tube Procedure

Applied as an EC broth confirmation of all positive presumptive tubes. . . . . ____

Place EC tubes in water bath within 30 min of transfers . . . . . ____

Incubate at 44.5°C ± 0.2°C for 24 hrs . . . . . ____

Gas production is positive test for fecal coliforms. . . . . ____

Calculate MPN based on combination of positive EC tubes . . . . . ____

b. Membrane Filter Procedure

Following filtration place MF over pad saturated with M-FC broth. . . . . ____

Place MF cultures in water-proof plastic bag and submerge in water bath within 30 min. . . . . ____

Incubate at 44.5°C ± 0.2°C for 24 hrs . . . . . ____

All blue colonies are fecal coliforms. . . . . ____

Calculate direct count in density per 100 ml . . . . . ____

56. Delayed-Incubation Coliform Test

After filtration, place MF over pad of M-Endo containing 3.2 ml of a 12% sodium benzoate solution per 100 ml of medium . . . . . ____

Addition of 50 mg cycloheximide per 100 ml of preservative medium for fungus suppression is optional . . . . . ____

Transport culture by mail service to laboratory within 72 hours . . . . ____

Laboratory	Location	Date

56. Delayed-Incubation Coliform Test (Continued)

Transfer MF cultures to standard M-Endo medium at laboratory. . . . . ____
Incubate at 35°C ± 0.5°C for 20 - 22 hr . . . . . ____
If at time of transfer, growth is visible, hold in refrigerator till end of work day then incubate at 35° overnight (16 - 18 hr period) . . . . . ____
Count sheen colonies, verify if necessary, and calculate direct count in coliform density per 100 ml . . . . . ____

57. Additional Test Capabilities

Fecal streptococci	_____	Method	_____
Pseudomonas aeruginosa	_____	Method	_____
Staphylococcus	_____	Method	_____
Salmonellae	_____	Method	_____
Biochemical tests	_____	Purpose	_____
Serological tests	_____	Purpose	_____
Other	_____	Purpose	_____

Laboratory Staff and Facilities

58. Personnel

Adequately trained or supervised for bacteriological examination of water . . . . . ____
Laboratory staff _____ (Total) Prep room staff _____ (Total)

59. Reference Material

Copy of the current edition of Standard Methods available in the laboratory . . . . . ____
State or federal manuals on bacteriological procedures for water available for staff use. . . . . ____

60. Physical Facilities

Bench-top area adequate for periods of peak work in processing samples. . . . . ____
Sufficient cabinet space for media and chemical storage. . . . . ____
Office space and equipment available for processing water examination reports and mailing sample bottles . . . . . ____
Facilities clean, with adequate lighting, ventilation and reasonably free from dust and drafts . . . . . ____

61. Laboratory Safety

Proper receptacles for contaminated glassware and pipettes. . . . . ____

Laboratory	Location	Date

61. Laboratory Safety (Continued)

Adequately functioning autoclaves with periodic inspection and maintenance. . . . . ____
Accessible facilities for hand washing . . . . . ____
Proper maintenance of electrical equipment to prevent fire and electrical shock . . . . . ____
Convenient gas and electric outlets. . . . . ____
First aid supplies available and not out-dated . . . . . ____

62. Remarks