

**RaPID Assay[®]
Environmental
User's Guide**

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SECTION 1 - INTRODUCTION

This manual is intended as a guide for first-time users of the RaPID Assay[®] kits and the associated equipment and as a reference for experienced users. In addition to information on setting up the required equipment and running SDI's RaPID Assays, this guide includes background information for applying the RaPID Assay system to environmental analyses.

For those users involved in petroleum hydrocarbon projects, an additional book entitled *Petroleum Hydrocarbon Detection Using RaPID Assays* is available from SDI (Part No. A00255). Due to the complex nature of petroleum contaminants, relating immunoassay results to project action levels typically requires approaches that differ from those outlined in this manual. *Petroleum Hydrocarbon Detection Using RaPID Assays* reviews the steps required to apply SDI's RaPID Assays to a petroleum hydrocarbon project.

Using this guide when beginning a project, first read the background section that pertains to the analyte(s) of interest (Section 2) and then using Sections 3-5 develop the sampling plan. Section 3 provides general QA/QC guidance, while Sections 4 and 5 describe an approach for determining an

appropriate project-specific cutoff concentration and optimizing the sample dilution scheme for that concentration. Examples of project-specific cutoff concentrations are described as screening levels in SW-846 Method 4000 (Section 2.21 of Method 4000).

Before running the first assay, read thoroughly those sections that refer to each piece of equipment to be used (Sections 6 - 14). Next, proceed to Sections 15 and 16 to run the assay. Section 17 is provided to assist the operator in resolving problems which might be encountered. For a more detailed explanation, refer to the operating manual for individual pieces of equipment and to the package insert for each assay kit.

For experienced users, the last section (18) in this manual can be directly referred to for brief instructions for the RPA-I and worksheets for common calculations. This section has been located at the end of the manual so the user can quickly find this frequently used material.

If any of the material contained in this manual is unclear or if problems are encountered, please call SDI's Technical Services at (800) 544-8881.

SECTION 2 - ANALYTE BACKGROUND

PCBs

Polychlorinated biphenyls (PCBs) are a class of 209 discrete chemical compounds (congeners) in which 1-10 chlorine atoms are attached to biphenyl. The individual congeners are substituted biphenyls which contain 1-10 chlorine atoms. PCBs were produced commercially as complex mixtures of congeners for a variety of uses, especially dielectric fluids in capacitors and transformers. PCBs sold in the United States were marketed under the tradename Aroclor®, from 1930 to 1977, for use in transformers, capacitors, printing inks, paints, dedusting agents, pesticides and other applications. The chemical and physical stability of Aroclors combined with their electrical insulating properties led to their commercial utility. Since PCBs do not readily degrade in the environment and are lipophilic, they persist and tend to bioaccumulate. Unfortunately, PCBs do not readily degrade and have been shown to be ubiquitous environmental pollutants occurring in most human and animal adipose samples, milk, sediment, and numerous other matrices.

In 1968, PCB-contaminated cooking oil was shown to cause an epidemic of severe acne among residents of a western province in Japan. Eventually, over 1000 people in this outbreak were diagnosed as having Yusho ("oil disease" in Japanese) in which acne is accompanied by other features including liver and nerve abnormalities in those who ingested the oil and low birth weights and hyperpigmentation in children born to exposed mothers. However, the toxicological data is confounded by the presence of other toxic compounds in the oil including polychlorinated dibenzofurans (PCDFs) that result from contamination and degradation of PCB. A similar

incident occurred in Taiwan in 1979. Over 2000 people, mainly school children near a food processing plant, were poisoned and diagnosed with Yucheng ("oil disease" in Chinese).

After the Yusho poisoning, concern about the widespread environmental occurrence, persistence, and health risk posed by PCBs resulted in regulation of the manufacture, processing, distribution, and use of PCBs in the United States under the Toxic Substance Control Act (TSCA). The U.S. Environmental Protection Agency (EPA) was given responsibility for enforcement of this act.

The EPA subsequently promulgated a series of rules, according to which: the discharge of PCB-containing effluents and the production of PCBs in the U.S. is prohibited; disposal of materials contaminated with PCBs is regulated; and use of PCB-containing materials still in service is restricted. PCBs are classified as a probable human carcinogen, Group B2, by the EPA (chemicals for which there is sufficient evidence of carcinogenicity in animals and inadequate data in humans). The EPA has established a Maximum Contaminant Level Goal (MCLG) of zero in drinking water. The Maximum Contaminant Level (MCL) and Practical Quantitation Limit (PQL) for drinking water have been set at 0.5 ppb of any Aroclor. In soil the EPA established 500 ppm as an incineration limit and 50 ppm for all Aroclors as the TSCA hazardous waste regulatory limit/landfill limit. Regulatory limits for soil remediation vary according to state and site, but, in general, they are 5 or 10 ppm for industrial restricted access areas and 1 or 2 ppm for residential access areas.

PENTACHLOROPHENOL

More pentachlorophenol (PCP, penta) is produced than any other chlorophenol, worldwide. Its pronounced biocidal activity led to its use in a great number of applications, most of which have become obsolete or prohibited. It has been used in the wood and paper industry as a fungicide to protect against fungal rot and as an insecticide for the control of termites and wood boring insects. Its primary applications are preservation of utility poles, railway ties, marine pilings and fence posts. In paper and pulp milling, it has been used as a slime reducer. Pentachlorophenol also has utility as a general herbicide, especially as a pre-harvest defoliant in cotton. Its past extensive use and its continuing application as a wood protectant have led to its ubiquitous presence in the environment.

Pentachlorophenol's high acute toxicity (estimated at 29 mg/kg) in man has led to a number of fatal poisoning incidents. As a carcinogen, studies on pentachlorophenol have divergent results. The U.S. EPA has classified

pentachlorophenol as a Group B2 contaminant, a probable human carcinogen based on sufficient evidence in animals and inadequate data in human studies. Short term exposure to high levels of pentachlorophenol can cause harmful effects to the liver, kidneys, skin, blood, lungs, nervous system and gastrointestinal tract.

The U.S. EPA has set a Maximum Contaminant Level (MCL) for pentachlorophenol in drinking water of 1 ppb, a Maximum Contaminant Level Goal (MCLG) of 0 ppb and a Practical Quantitation Limit (PQL) of 1 ppb. Pentachlorophenol is a regulated chemical, is included in the EPA's Extremely Hazardous Substance List, and is reported in the EPA's Toxic Substances Control Act Inventory. Recently, pentachlorophenol regulations under the Resource Conservation and Recovery Act (RCRA) have been created specifically for wood treatment facilities. Pentachlorophenol has been detected at 84 of the 1,177 National Priorities List (NPL) hazardous waste sites in the United States.

Total BTEX/TPH (PETROLEUM HYDROCARBONS)

There are a variety of fuel-derived hydrocarbons that contaminate the environment. Small aromatic hydrocarbons, which include BTEX (benzene, toluene, ethylbenzene and xylenes) and semi-volatile compounds (naphthalene and phenanthrene), are prevalent contaminants at hazardous wastes sites across the United States as a result of spillage of hydrocarbon fuels and leaking underground storage tanks. It has been estimated that there are 2 to 5 million underground storage tanks containing petroleum hydrocarbons or chemical present in the United States. Suspected contamination sites must be evaluated to map out a contamination plume, to establish if the plume is migratory, to determine if remediation is needed or to determine the effectiveness of a remediation effort. Identifying the presence of hydrocarbon contamination is a high priority during site investigation and may result in a site being listed on the National Priorities List (NPL) established by the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA or Superfund). Monitoring exposure via drinking water, especially groundwater, is the stated purpose of much of the hydrocarbon and underground storage tank regulations.

The collection and analysis of soil and groundwater samples have become the most widely practiced methods of site assessment and have gained wide acceptance of regulatory agencies. Petroleum hydrocarbon chemicals can occur in the liquid, dissolved, adsorbed, or vapor phase. Chromatographic effects such as differential multiphase partitioning, selective solubilization and other "weathering" effects are characteristic difficulties encountered during the measurement of hydrocarbon contamination. Accurate and precise characterization of a contaminated region of interest may be difficult due to inherent uncertainty in measuring and interpreting results obtained while measuring the complex mixture of for volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) found in petroleum products.

Soil sampling is a critical element in characterizing the magnitude and extent of contamination despite the high

degree of variability in results due to spatial and temporal variation in soil properties and the small fraction of the total volume of a soil at a site that is sampled.

Hydrocarbons may migrate long distances along subsurface structures such as sewer lines and other utility trenches. In addition, a number of mechanisms and factors affect the behavior of petroleum hydrocarbons in unsaturated soil. These factors include transport of chemical compounds by gas or liquid diffusion, hydrodynamic dispersion, sorption, volatilization/dissolution, abiotic degradation and biodegradation.

Current EPA field sampling guidelines (SW-846) for the determination of VOCs in soil specify that subsamples be shipped to the laboratory in bottles that have been filled to capacity (i.e. no headspace) and stored at 4°C for no more than 14 days.

To effectively apply any analytical method, its characteristics should be understood. Chromatographic methods used in conjunction with soil sampling techniques can measure individual volatile compounds (e.g., BTEX), or the sum of individual chemical concentrations (e.g., gasoline range organics [GROs], diesel range organics [DROs], or Method 8015M).

Immunoassay techniques measure the total response of the antibodies employed to small aromatic hydrocarbons. Therefore, selection of the proper RaPID Assay for petroleum hydrocarbons requires an understanding of the nature of the hydrocarbon contamination, regulatory requirements driving the monitoring activity, and sensitivity objectives (i.e., detection levels). Use of the proper RaPID Assay for screening hydrocarbons will result in faster results, allow better coordination of field activities (e.g., proper characterization of soils, locating monitoring wells, movement of contaminated soil or improving the efficiency of remediation activities), and minimize potential loss of volatiles.

See the *Petroleum Hydrocarbon Detection Using RaPID Assays* booklet for further information on this topic.

POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs)

Polynuclear or polycyclic aromatic hydrocarbons (PAHs) are a group of semivolatile compounds composed of two or more fused aromatic rings. The U.S. EPA has identified 16 unsubstituted PAHs as priority pollutants.

Polynuclear Aromatic Hydrocarbon U.S. EPA Priority Pollutants

Two-ring

Naphthalene

Three-ring

Acenaphthalene
Phenanthrene
Anthracene
Fluorene
Acenaphthylene

Four-ring

Benzo[a]anthracene
Pyrene
Fluoranthene
Chrysene

Five-ring

Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benzo[a]pyrene
Dibenzo[a,h]anthracene

Six-ring

Indeno[1,2,3-*cd*]pyrene
Benzo[*g,h,i*]perylene

Some of the four, five and six-ring PAHs such as chrysene, benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene are considered to be probable or possible human carcinogens. Benzo[*a*]pyrene is the most potent carcinogen among the PAHs. PAHs are introduced into the environment as a product of natural and fossil fuel combustion; volcanic

eruptions and forest fires are the major natural sources. As a source of environmental contamination, PAHs are a serious problem at manufactured gas plants (MGP), coking operations, wood preserving sites that use creosote as a preservative and petrochemical waste disposal sites. The large number of these sites that are contaminated by PAHs in soil and groundwater has led federal and state agencies to mandate their clean-up. These agencies have set various regulatory levels for PAHs in soil; however, the usual concentrations of interest are 1 ppm and 10 ppm total PAHs. Accurate determination of the PAH content of soils suspected of contamination is necessary to make appropriate decisions regarding site cleanup and remediation.

PAHs have been detected in surface waters of the United States because of runoff from industrial and municipal sources. Due to the hydrophobicity and strong affinity for particulate matter of most PAHs, concentrations are usually higher in the sediment than surface and ground water. PAHs are also found as a component of petroleum products especially creosote, home heating oil, fuel oil and, to a lesser extent, diesel fuel, kerosene, jet fuel, and gasoline. When screening a site for PAH contamination, use of the RaPID Assay kits provides investigators with a single value that represents a sum of the antibody reactivity with the recognized PAHs rather than a measurement of the 16 individual chemicals.

See the *Petroleum Hydrocarbon Detection Using RaPID Assays* booklet for further information on this topic.

TNT

TNT is the common name for 2,4,6-trinitrotoluene, the most widely used military high-explosive. It has found wide application in shells, bombs, grenades, demolition and propellant compositions. TNT is among the least impact and friction sensitive of the high explosives but can be exploded on impact by a detonator mechanism. It is used as a standard explosive against which all other military high explosives are rated. The presence of TNT and other explosives in water and soil is one of the most serious environmental problems facing the military sites where munitions were manufactured, stored or used.

TNT is also considered highly toxic, mutagenic and carcinogenic in bacterial and animal tests (USEPA, 1989). TNT is released to the environment as a result of open detonation and burning techniques used in the disposal of munitions. Historically, large quantities of aqueous TNT effluents were discharged at military sites where munitions were manufactured, stored or used. As much as one half million gallons of TNT waste water are

generated per day by a single TNT production facility (Hartley et al., 1981). "Red water" and "pink water" found at manufacturing, assembly and packing sites are due to TNT and other nitrated compounds and are classified as hazardous waste by the EPA and cannot be discharged into streams.

TNT has been identified in at least 19 of 1,300 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL). The U.S. EPA has assigned TNT a carcinogenic classification of C, which indicates that it is a possible human carcinogen. The Drinking Water Equivalent Level (DWEL), a lifetime exposure at which adverse health effects would not be expected to occur, is 20 ppb. The Lifetime Health Advisory for TNT in drinking water has been set at 2 ppb (USEPA, 1989). Soil clean-up levels vary from site to site but generally will be in the range of 1 to 30 parts per million.

CARCINOGENIC PAHs

Polynuclear or polycyclic aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings. The U.S. EPA has identified 16 unsubstituted PAHs as priority pollutants.

The seven (7) PAHs that are typically considered to be probable or possible human carcinogens are benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene. Benzo[a]pyrene is the most potent carcinogens among the PAHs. Under certain circumstances site remediation can be based on carcinogenic PAH levels and site risk assessment is usually based on the carcinogenic PAH contamination.

Carcinogenic PAHs are introduced into the environment as a product of natural and fossil fuel combustion. Volcanic eruptions and forest fires are the major natural sources of PAHs. However, the ubiquitous nature of PAHs can be attributed to fossil fuel combustion sources such as automobiles, coking plants, asphalt production and manufacturing facilities.

As a source of environmental contamination, PAHs are a serious problem at manufactured gas plants (MGP), coking operations, wood preserving sites that use creosote as a preservative and petrochemical waste disposal sites. The large number of these sites which are contaminated by PAHs in soil and groundwater as led to federal and state agencies to mandate their clean-up. The federal and state agencies have set various regulatory levels for PAHs in soil, however the usual concentrations of interest are below 1 ppm for individual carcinogenic PAHs.

In drinking water, benzo[a]pyrene has been classified as a Group B2 carcinogen (probable human carcinogen based on a combination of sufficient evidence in animals and inadequate in humans). The U.S. EPA has established a Maximum Contaminant Level Goal (MCLG) of 0 ppb, a Practical Quantitation Limit (PQL) and Maximum Contaminant Level (MCL) of 0.2 ppb in drinking water (USEPA, 1994). The major source of benzo[a]pyrene in drinking water is leachate from coal tar lining and sealants in water storage tanks and leachate to aquifers from coal tar pits at manufactured gas plant (MGP) sites.

See the *Petroleum Hydrocarbon Detection Using RaPID Assays* booklet for further information on this topic.

CYCLODIENES

The cyclodiene insecticides encompass a large group of polychlorinated cyclic hydrocarbons with endomethylene bridged structures. Among them are two pairs of stereoisomers: aldrin and isodrin, dieldrin and endrin. Other cyclodiene compounds of interest include heptachlor, chlordane, endosulfan and isobenzan.

Toxicity and storage (bioaccumulation) vary widely among the cyclodienes and even among the stereoisomers. For example, endrin appears to rapidly decrease in the body during the first few days after poisoning. The acute toxicity of the dieldrin is less than that of endrin, but dieldrin is so avidly stored that traces of it may be found

in almost everyone in the general population. Bioaccumulation of the highly lipophilic cyclodienes, such as dieldrin and heptachlor epoxide, has resulted in widespread distribution in human fat and milk. Fish have been found to have up to 300,000 fold accumulation of these compounds through exposure to water

Until recent discountinances of production and limitations on usage, the cyclodienes most commonly used in the United States were heptachlor, chlordane, aldrin, and dieldrin. Currently, the use of most cyclodiene insecticides is also severely limited or banned in many countries.

SECTION 3 - QUALITY CONTROL AND SAMPLING PLANS

QUALITY ASSURANCE / QUALITY CONTROL (QA/QC) PROGRAM

A QA/QC program specifies analytical procedures and techniques for evaluating the accuracy and precision of analytical data in order to establish the quality of the data that will be used for management decisions. It can also provide an indication of the need for corrective action and determine the effect of such actions. The U.S. EPA in *Test Methods for Evaluating Solid Wastes* (SW-846) has recommended that a QA/QC program be structured in two major sections. The first, a program plan, serves as an operational charter for the laboratory and sets up basic laboratory policies. The second section is a project specific plan which provides the specific details for sampling, analysis, and data evaluation. The essential elements of a QA Project Plan (QAPP) are:

- Title Page
- Table of Contents
- Project Description
- Project Organization and Responsibility (including an organization chart)
- QA Objectives
- Sampling Procedures
- Sample Custody
- Calibration Procedures and Frequency
- Analytical Procedures
- Data Reduction, Validation, and Reporting
- Internal Quality Control Checks
- Performance and System Audits
- Preventive Maintenance
- Specific Routine Procedures Used to Assess Data Precision, Accuracy, and Completeness
- Corrective Action

- Quality Assurance Reports to Management

Additional guidance on preparing the QAPP is provided by the EPA's Quality Assurance Management staff.

In the U.S. EPA's *Quality Assurance/Quality Control Guidance for Removal Activities* from the Office of Emergency and Remedial Response, the QA Project Plan is further subdivided into a generic "Branch QA Project Plan" and a site-specific "Sampling QA/QC Plan." The Sampling QA/QC Plan (OSWER Directive 9360.4-01 Guidance) should contain the following sections:

- Title Page
- Background
- Data Use Objectives
- Quality Assurance Objectives
- Approach and Sampling Methodologies
- Project Organization and Responsibilities
- Quality Assurance Requirements
- Deliverables
- Data Validation

Whenever immunoassays are specified as analytical procedures, careful consideration should be given to the characteristics of the method. As stated in OSWER Directive 9360.4-01 Guidance, "the quality of the data is determined by its accuracy and precision against prescribed requirements or specifications, and by its usefulness in assisting the user to make a decision or answer a question with confidence." Immunoassay methods have performance characteristics that allow them to be effectively employed in many projects. They are particularly useful when results are required rapidly.

QA/QC METHODOLOGY

DATA USE AND DATA QUALITY (QA) OBJECTIVES:

The purpose of the data collection should be clearly understood before sampling begins. The intended use affects the choice of analytical method as well as the QA objective.

Immunochemical methods are used commonly for QA1 and QA2 but they can be used, in some circumstances, to achieve QA3 objectives. The EPA has described three primary data quality levels.

Level QA1. The QA1 objective is intended as a screen to afford a quick, preliminary assessment of site contamination. The objective is to provide an indication of contamination and is not a definitive identification of pollutants nor their concentration. **This data objective can be achieved by immunoassay analysis to determine the extent and degree of contamination relative to concentration differences, delineation of pollutant plume in ground water, monitoring well placement, and preliminary identification and quantitation of pollutants.**

Level QA2. The EPA calls Level QA2 a verification objective. This objective is intended to give the user a level of confidence in a select portion of the preliminary data by verification with a more rigorous analyte specific method. This objective should be applied to verification of pollutant plume definition in ground water, verification of health and safety assessment, verification of pollutant identification, and verification of cleanup. **This data objective can be achieved with immunoassay field screening methods after verification of at least 10 percent of the samples with an EPA-approved method.**

Level QA3. QA3 is a definitive objective used to assess the accuracy of the concentration level as well as the identity of the analyte of interest. This quality objective is intended to give the user a level of confidence for a select group of critical samples so that decisions can be made regarding treatment, disposal, site remediation, and other

significant decisions where an action level is of concern. It generally involves second method confirmation on 100 percent of the critical samples. At this level analytical error is quantified and monitored.

To summarize, immunochemical methods are used commonly for QA1 and QA2 but they can be used, in some circumstances, to achieve QA3 objectives.

APPROACH AND SAMPLING METHODOLOGIES:

General Considerations. The first step in any successful analysis is the collection of a sample. The selection of sampling sites, frequency of sampling, number of samples, measurement of physical and chemical parameters of the sample, potential for cross contamination, and the overall statistical design of sampling methods are all important factors to consider.

A sampling plan should be based on regulatory and scientific objectives. A good sampling plan will define representative samples and assess the sampling and analytical precision. Taking a preliminary estimate of the mean and the variability of the sample result, the appropriate number of samples can be calculated by a statistical comparison to the regulatory threshold and the desired level of confidence. It is prudent to collect a greater number of samples than preliminary estimates indicate since a poor preliminary estimate could result in an underestimate of the appropriate number of samples to collect. Often it is possible to collect and store extra samples for later analysis if needed.

A variety of sampling plans can be developed dependent on the project objective, the nature of the sample and knowledge of the distribution of the contaminant. Sampling accuracy is usually achieved by some form of random sampling. Sampling precision is most commonly achieved by taking an appropriate number of samples. If the site to be sampled is randomly heterogeneous or if little or no information is available concerning the distribution of the contaminants, sampling accuracy and precision can usually be

achieved by simple random sampling. As more information is accumulated for the contaminants of concern, other sample approaches can be applied including stratified random sampling, systematic random sampling and authoritative sampling.

Immunochemical Methods. An immunoassay method provides results more rapidly and at a lower cost than traditional methods. Immunochemical analysis can be employed to guide field work and sampling efforts including: determining the vertical and horizontal extent of soil contamination, tracking contamination plumes in groundwater, and determining surface water contaminations. Due to the cost-effectiveness of immunochemical methods, efforts to assess the extent of a contamination can be enhanced by collecting more closely-spaced samples. This allows a more detailed determination of contaminant location. A particularly efficient approach is to collect initial samples from relatively large grids or strata, and then, when the initial results are available, sample areas of greatest interest in smaller grids or strata to obtain a more detailed assessment. This iterative process can be employed to obtain detailed information over large areas.

The availability of rapid results from immunoassays makes it useful in monitoring the effectiveness of remediation techniques to reduce or eliminate a contamination. The sampling plan in these applications can employ samples taken more frequently, approaching a "real time" monitoring of the remediation process to determine if the contaminant concentration in soil or water samples exceeds site-specific action limits.

Sampling Equipment & Storage. The choice of sampling equipment and sample containers will depend upon the contaminant of interest and site considerations. In making decisions about sampling equipment, the user needs to consider:

- 1) potential interactions between the sampling equipment or container with the analytes of interest including losses by adsorption, volatilization, or contamination caused by leaching from the containers or sampling devices;
- 2) cleaning procedures to minimize sample contamination and cross contamination between samples; and
- 3) analyte-specific properties that may dictate the use of optimum equipment or techniques such as minimizing agitation for volatile compounds.

Once the sample has been collected it must be stored to maintain the chemical and physical properties that it possessed at the time of collection. Specifications for sample containers, sample preservation, and sample holding times are often included in EPA or state regulations.

To assist the user, SDI provides collection devices and sample containers optimized for use with the RaPID Assay system. The Soil Collection Kit includes a collection device and glass vials with Teflon lined caps for storage of the extract. The performance of the collection device and the stability of the extract have been characterized by SDI for each analyte and reported in their respective Sample Extraction Kit package inserts. Also provided in the Soil Collection Kit are labels for the collection devices and extraction collection vials to aid in chain of custody documentation. A summary of storage information is given in the following table (see the *Petroleum Hydrocarbon Detection Using RaPID Assays* book for storage information for petroleum hydrocarbon methods).

STORAGE OF SAMPLES

Sample	PCBs	Pentachlorophenol	TNT	Cyclodienes
Soil*	14 days at 4°C	14 days at 4°C	14 days at 4°C	14 days at 4°C
Water*	7 days at 4°C	7 days at 4°C	7 days at 4°C	7 days at 4°C
Soil Extract	7 days at RT	7 days at RT	14 days at RT	14 days at RT
Diluted Extract	7 days at RT or 1 month at 4°C	7 days at RT or 1 month at 4°C	7 days at RT or 1 month at 4°C	7 days at RT or 1 month at 4°C

* Recommendations consistent with EPA SW-846 holding times

MEASUREMENT QUALITY ASSURANCE REQUIREMENTS:

Level QA1. The requirements for Level QA1 can be readily met using the RaPID Assay system. The following requirements apply:

- A. **Sample documentation.** The sample location, depth and matrix can be documented along with sample collection time, date and field analysis time and date.
- B. **Instrument calibration data or a performance check of a test method.** SDI recommends that the Positive Control provided with each RaPID Assay kit be included in an analytical batch. Results obtained for the Positive Control should be within a previously established range. Guidance on obtaining this acceptable range is provided in the RaPID Assay kit package insert in the Quality Control section. Proficiency Samples can be obtained from SDI at different analyte concentrations in order to satisfy project-specific requirements.
- C. **Detection limit.** The detection limit should be determined to ensure that RaPID Assay kits are appropriate for regulatory or project-specific requirements. Detection limits are discussed in the "Selecting a Cutoff Concentration" section of this guide.

If desired, the detection limits of a RaPID Assay method can be determined experimentally. In SW-846, method detection limit is defined as the analyte concentration three standard deviations above the average noise level of the signal. A similar estimate can be made using an immunochemical method by estimating the

mean and standard deviation at zero analyte concentration. Using these estimates the detection limit can be determined as the concentration equivalent to the absorbance calculated for the mean zero absorbance minus three times the zero concentration standard deviation (mean - 3 X SD). The estimates given in SDI literature are generally a more conservative estimate. Alternatively, the detection limit can be monitored by establishing an acceptable range for the ratio between the absorbance of the zero standard and Standard 1.

- D. **Other controls measures for consideration.** Although not required in EPA Level QA1 plans, SDI recommends that:
 - The raw data (i.e. the printout from the RPA-I , or RPA-VI which can be copied for a permanent record) and calculations used for final results be documented.
 - The three standard levels and the zero provided with each RaPID Assay kit be run in duplicate in each analytical batch.
 - An uncontaminated sample from each site matrix be analyzed each day to document possible matrix interference.
 - Sample duplicates be performed on at least one out of every twenty samples to document method repeatability.

Level QA2. In addition to the requirements listed for Level QA1, the following are required for QA2:

- A. **Chain of custody.** This requirement is optional for field screening. The typical components of a chain of custody system are

sample seals, a field logbook, chain of custody record, and sample analysis request sheet. In a chain of custody system the possession and handling of samples should be traceable from the time of collection through analysis and final disposition. Chain of custody documentation should be prepared for samples used in confirmation testing.

- B. **Sample holding times.** Since immunochemical methods are often carried out in the field, samples are typically analyzed shortly after collection and sample holding times are brief. As previously described sample holding times for the extracts in the RaPID Assay system are given in the Sample Extraction Kit package insert and summarized in a previous section of this manual. Guidance for holding times for the EPA methods is given in *Quality Assurance/Quality Control Guidance for Removal Activities and Test Methods for Evaluating Solid Wastes* (SW-846).
- C. **Initial and continuing instrument calibration data.** The calibration of a photometer (RPA-I, III or VI) can be verified by using solutions of potassium dichromate or methyl orange at established absorbances. These solution absorbances can be determined in a reference spectrophotometer. The RPA-I has built-in functions to aid in the evaluation of these solutions (See the RPA-I Operating Manual Section 3 - Operating Procedures/Menus -Special Functions - Linearity Test and Tube Repeatability)
- D. **Method, rinsate, and trip blank data.** A method blank can be prepared by processing the RaPID Prep Extraction Solution through the entire sample preparation scheme and analysis. It is recommended that one method blank be performed daily. Rinsate blanks are only required if dedicated sampling tools are not used. If the Soil Collection Device is used as the sampling tool a rinsate blank is not needed. A trip blank consisting of vials filled with distilled/deionized water should be included for volatiles transported by cooler for confirmation analysis.

- E. **Confirm 10 percent of the results from the screening with an EPA-approved (e.g., chromatographic) method.** Samples collected for confirmation should represent various analyte concentration levels (above and below the action level) and various site locations. Documentation of the confirmatory analysis such as gas chromatograms and mass spectra should be prepared.
- F. **Performance evaluation samples are optional.** Performance evaluation samples consisting of the appropriate matrix containing an independently determined analyte concentration are often commercially available. Contact SDI Technical Services for further information. It is recommended that one performance evaluation sample be run daily.
- G. **Other control measures for consideration.** Although not required in EPA Level QA2 plans, SDI recommends:
- Matrix spikes be included. To perform a matrix spike, add a predetermined quantity of stock solution of the analyte of interest to a sample matrix prior to sample extraction. Contact SDI Technical Services for further information.
 - The raw data (i.e. the printout from the RPA-I or RPA-III) and calculations used for final results be documented.
 - The three standard levels and the zero provided with each RaPID Assay kit be run in duplicate in each analytical batch.
 - An uncontaminated sample from each site matrix be analyzed each day to document possible matrix interference.
 - Sample duplicates be performed on at least one out of every twenty samples to document method repeatability.

DATA VALIDATION:

Level QA1. QA1 data need only be evaluated for calibration and detection limits criterion.

Level QA2. The results of 10% of the samples in the analytical package should be evaluated for

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QA & SAMPLING PLANS**

all of the documentation elements. The holding times, blanks, and detection limits should also be reviewed.

OTHER QA/QC CONSIDERATIONS:

- Carefully and thoroughly read the entire package insert provided with the kits.
- Use replicate analyses when results indicate concentrations near the action level.
- Do not use kits past their expiration date.
- Do not mix reagents from one kit with another kit.
- Kits should be stored as indicated in instructions (generally 2-8°C for RaPID Assay kits and 2-30°C for RaPID Prep

Extraction kits) and extended transit time at ambient conditions avoided.

- Appropriate training of operators is critical in obtaining reliable results. Materials (e.g. videotape, proficiency samples, written information) and assistance are available from SDI.
- Those responsible for management decisions based on immunoassay results should understand the technology, particularly the issues of specificity and cross-reactivity.
- Additional information on quality control of immunoassays can be obtained from SDI Technical Report T00029.

SECTION 4 - SELECTING A CUTOFF CONCENTRATION

GENERAL

Depending on the project, either quantitative or qualitative results may be required from an analytical method. For example, measurements made to satisfy RCRA requirements using SW-846 methods specify that immunoassay is suitable only for sample screening (qualitative or semi-quantitative analysis). That is, the concentration of contaminant in a sample is evaluated relative to an action or regulatory level and the sample is defined as above (positive) or below (negative) the action level for qualitative analysis or above, below, or between defined levels for semi-quantitative analysis. For use of RaPID Assay kits as SW-846 methods see SDI Technical Services Bulletins T00094, T00105 and T00106.

Due to the complex nature of petroleum contaminants, relating immunoassay results to project action levels typically require approaches that differ from those outlined in this manual. The book *Petroleum Hydrocarbon Detection Using RaPID Assays* reviews the steps required to apply SDI's RaPID Assays to a petroleum hydrocarbon remediation project and is available from SDI (Part No. A00255).

The action level for projects vary with analyte, local regulations and project-specific data quality objectives (DQOs). The RaPID Assay System, provides three standard levels for the generation of a calibration curve. This allows the user to select a cutoff concentration appropriate for the particular project and to modify the dilution scheme and optimize the method for that particular concentration. This section provides guidance on factors to consider when selecting the appropriate cutoff for a project. The next section provides instructions on how to perform the optimum dilution of sample to detect the analyte at the cutoff concentration.

ACTION LEVELS

In most projects an Action Level is determined from regulatory and safety considerations. The

concentrations of chemical contaminants cannot be equal to or exceed this level. Since there are a number of factors that affect a result from an analytical method, the highest concentration that would be judged as non-hazardous (negative) would be somewhat less than the Action Level. The difference between this level, which will be referred to as the "Cutoff" and the Action Level will be affected by a number of factors.

FACTORS AFFECTING THE CUTOFF

REACTIVITY:

All immunoassays rely on antibodies as the critical analytical reagent. Antibodies, large protein molecules known as immunoglobulins, are produced by the immune system of animals in response to foreign substances (antigens). Antibodies have the ability to specifically and tightly bind antigens. These are the properties that are exploited in an immunoassay.

Specificity describes the ability of an analytical method to distinguish between true (or specific) and interferences (or non-specific) results. Immunoassays can be developed to have different levels of specificity depending on the intended application of the immunoassay. Immunoassays can be very specific, detecting only one chemical compound, or they can be made less specific so that related compounds can be detected. The level at which related compounds are detected in an immunoassay is referred to as "cross-reactivity."

The characteristic specificity of an immunoassay is an important factor in the determination of a cutoff concentration. An immunochemical method developed for a class of compounds (e.g., PCBs) may be more sensitive to some members of the class (e.g., Aroclor 1254) than others. If a site has been characterized as being contaminated with predominantly one member of the class, this should be taken into account when determining the cutoff concentration. Otherwise, the method should be calibrated with the component giving the greatest response to the assay (i.e., Aroclor

1260 for PCBs). In the RaPID Assay package insert for each method, data are provided that characterize the reactivity of each specific contaminant. These data represent the results obtained under a particular set of conditions and should be used as estimates of reactivity relative to the kit calibrators. It should be recognized that as conditions change (temperature, timing, etc.) the apparent reactivity of a class of compounds can change. Sometimes these changes can be significant (50% or more). Therefore, whenever possible, the assay should be directly calibrated with the compound of interest for greatest analytical accuracy.

The issue of reactivity becomes more complicated when dealing with mixtures of reactive components. When the components are present as a mixture, their composite reactivity with the assay is not a simple sum of the individual reactivities or concentrations. Consequently, even if the user knows the exact nature and component composition of the contaminant it may be difficult to predict the reactivity of the assay with the contaminant mixture. This situation is further complicated because important factors such as formulation and aging effects are rarely known with any certainty. In projects where the composition of the contaminant has been quantified by a chromatographic method, this analysis can be used to assist the interpretation of the immunoassay results. In general, when analyzing multi-analyte mixtures, the immunoassay will provide qualitative information useful for screening for the presence or absence of the contaminants. Any measurements should be interpreted relative to other samples contaminated with the same mixture of constituents at the same site.

EXTRACTION EFFICIENCY:

When using an immunoassay for water samples, the extraction efficiency is not a factor since water is assayed directly. However, to analyze soil samples by immunoassay, the contaminant must be extracted from the soil matrix. Ideally, an extraction method should be effective on all soil samples taken from a site. However, since soils vary greatly in physical, chemical, and

mineralogical characteristics, the degree of analyte retention and the extractability can vary dramatically among different soils. Soils higher in clay and/or organic matter content generally have a greater cation exchange capacity and thus greater adsorption capacity for contaminants. As a result, the recoveries from soil may vary depending on soil type, retention mechanism, solvent and extraction apparatus used, length of extraction period, amount of agitation and levels of potentially interfering substances in the soil.

The extraction efficiency can influence the selection of a cutoff concentration. The user should select a lower cutoff concentration in projects where the extraction is less efficient (i.e., recoveries are lower), even if the regulatory action level is the same. To aid the user in establishing the extraction efficiency, estimates based on experimental studies are provided in the SDI RaPID Prep Sample Extraction kits (see *Expected Results* section under Recovery) and summarized in the following table:

Method	Average Extraction Efficiency (%)
PCB (as Aroclor 1254)	85
Pentachlorophenol	70
TNT	97
Cyclodienes (as dieldrin)	101

These estimates are based on a limited number of soil types spiked with a particular compound using the kit extraction protocol. It is recommended that the user establish an extraction efficiency for each project, analyte or soil type. This estimate can be made by testing recovery of a matrix spike using a soil sample from the site.

ANALYTICAL CONFIDENCE:

In all analytical methods, whether they are used for individual analytes or mixtures of analytes, a number of independent measurements of a single sample will produce a range of results. These results can be described in terms of bias and variability. Ordinarily, results from repeated independent measurements will be "normally" distributed (see Figure 1).

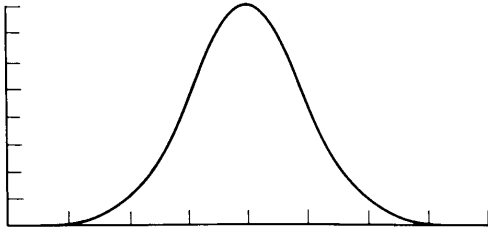


Figure 1 - Normal Distribution Frequency Plot

If the results are normally distributed and the variability of the method has been characterized, a user can predict how often (i.e., the frequency) that a result will occur above or below a particular known analyte concentration. When choosing a cutoff value it is important to understand the probability of obtaining positive and negative results at various concentrations relative to the action level. *If one chooses a cutoff concentration too close to the action level, incidence of false negative results will be high. On the other hand, if the cutoff is much lower than the action level the frequency of false negatives will decrease but the incidence of false positives (positive results for concentrations below the action level) will increase.*

To assist the user in determining the appropriate *Analytical Confidence Factor*, SDI has developed tables for each analyte and sample matrix that estimate the frequency of positive and negative results at various sample concentrations. These estimates have been statistically derived from the precision that has been observed for most methods at approximately the midpoint of the calibration curve. The mid-point of the calibration curve is generally the most precise region for obtaining immunoassay results. If a different cutoff concentration is chosen, the tables provided may no longer be appropriate. *Consult SDI's Technical Services for assistance in developing a strategy for screening difficult samples or when data quality other than screening results are required.*

CHOOSING A CUTOFF

A number of approaches can be taken to determining the concentration at which immunoassay results will be considered positive. In this section three approaches will be presented

in order of analytical preference. Some approaches may not be feasible due to the availability of analysis or materials. Approaches for petroleum hydrocarbons are reviewed in the manual, *Petroleum Hydrocarbon Detection Using RaPID Assays*, available from SDI.

METHOD 1 - USING A SITE SAMPLE:

In many cases initial site assessment activities will include analysis of one or more samples likely to be contaminated at concentrations above the established action level by an accepted traditional chromatographic method. Assuming this sample is representative of the site contamination and the chromatographic procedure is under statistical control (QA Level 3), results for this sample can be used to establish a cutoff concentration for the immunoassay procedure. The preferred approach is to analyze a homogeneous split soil using the chromatographic procedure and the immunoassay kit according to the specific steps of each method including dilution procedures. The extract can then be diluted to produce a concentration corresponding to the immunoassay cutoff point. This diluted extract is then run as a single point calibrator during each assay run. Samples yielding results higher than the diluted calibrator should be considered positive and those giving a lower result, negative. Using this technique the immunoassay results are adjusted for extraction efficiency and contaminant reactivity on a run by run basis.

An example of this method of standardization might be a site to be assessed for PCB contamination. In this example, a soil sample from the site was analyzed using Method 8080 and a result of 230 ppm was obtained. Because the site action level of 10 ppm had been established during the DQO process, a 1:23 further dilution of the sample extract should be prepared according to the immunoassay extraction method and used as a calibrator for the PCB immunoassay. Sample extracts resulting in absorbance values greater than the absorbance value obtained for this calibrator would be considered negative while those giving lower absorbance values would be considered positive for PCBs.

SECTION 4 SELECTING A CUTOFF

Alternatively, the appropriately diluted sample extract can be assayed by the immunoassay and a corresponding immunoassay concentration unit value established. Continuing the example given above, the sample extract after the additional 1:23 dilution was assayed using the immunochemical method and due to the particular Aroclor mixture, produced a result of 5 ppm PCBs when compared the kit standards. Samples can then be analyzed and their values compared to this result to classify as positive or negative, i.e. results greater than 5 ppm would be classified as positive. This technique will correct for extraction efficiency and contaminant reactivity on a one time basis. This approach is particularly useful when the analyte concentration in the sample is unstable making repeated use of the sample to calibrate the immunoassay unreliable. Using this method of calibration does not account for the effect of operator training and assay conditions on measurement accuracy. Although it is better to run a site specific calibrator in each assay run, this technique of assigning a site calibration to the kit standard is often more practical when the quantity of diluted extract is limited and a reference material is not available.

METHOD 2 - CALIBRATING WITH A SITE SPECIFIC REFERENCE MATERIAL:

In some cases a qualified reference material can be obtained that reflects the contamination found at a site. In these cases a standard can be prepared from this material in order to calibrate the immunoassay during each run. For example, when a contaminant has been previously characterized as Aroclor 1248, an analytically pure reference standard of Aroclor 1248 can be diluted in PCB Sample Diluent to the Action Level and used as a calibrator for the immunoassay in place of the Aroclor 1254 standard. For assistance in determining the availability of reference materials and preparation of site-specific standards, contact SDI Technical Services.

METHOD 3 - CALCULATING FROM ASSAY PERFORMANCE DATA:

The following protocol describes a method for mathematically estimating a cutoff concentration for a project. This technique will be difficult to perform when mixtures of reactive compounds are anticipated and is not recommended for use with the petroleum hydrocarbon methods (refer to the manual, *Petroleum Hydrocarbon Detection Using RaPID Assays* for further guidance). A worksheet follows this protocol to aid in the calculation of a cutoff concentration. Refer to the appropriate method tables which follow the worksheet for analyte specific information.

1. Record the *Action Level (A)* of the analyte of interest required for the specific project.
2. Determine and record the *Reactivity Factor (B)* from the Reactivity Table provided for the specific analyte (Table 1). If a mixture of reactive compounds are expected, conservatively use the least reactive compound for calculations. This may result in a lower cutoff than necessary increasing the likelihood of false positive results, but it will minimize the potential for false negative values.
3. Record the *Extraction Recovery Factor (C)*. This factor may be estimated from the information provided in the appropriate RaPID Prep Sample Extraction kit or from a site spiked matrix recovery experiment.
4. Choose and record an *Analytical Confidence Factor (D)* from the analyte specific information that follows the worksheet (Table 2).
5. Calculate the *Cutoff Concentration* to be applied to the assay results (after correction for dilution) from the following equation:
$$\text{Cutoff} = A \times B \times C \times D$$
6. Using the calculated concentration as the *Cutoff Concentration*, determine the required dilution and perform the required analysis by following the instructions in the next section, *Selecting the Range (Dilutions)*.

WORKSHEET

Required Action Level _____ (A)

Reactivity Factor _____ (B)
(from Table 1)

Extraction Recovery Factor _____ (C)
(see p. 4.2)

Analytical Confidence Factor _____ (D)
(from Table 2)

Cutoff Concentration _____ (E)
A Δ B Δ C Δ D

Example -

To optimize the PCB RaPID Assay system for detection Aroclor 1248 at 10 ppm in soil:

<i>Required Action Level</i>	10 ppm as <u>Aroclor 1248</u> (A)	An <i>Action Level</i> of 10 ppm of Aroclor 1248 is assumed for this example. See <i>Action Level</i> in text above.
<i>Reactivity Factor</i> (from Table 1)	<u>0.85</u> (B)	Since the PCB to be detected in this example is Aroclor 1248, the <i>Reactivity Factor</i> obtained from Table 1 of the PCB RaPID Assay Performance Characteristics is 0.85. Using this factor allows Aroclor 1254 calibrators to be used in assessment of the cutoff concentration.
<i>Extraction Recovery Factor</i>	<u>0.85</u> (C)	The <i>Extraction Recovery Factor</i> of 0.85 is obtained from the RaPID Prep Sample Extraction kit package insert. Preferably, this factor would be obtained from a spiked matrix determination.
<i>Analytical Confidence Factor</i> (from Table 2)	<u>0.80</u> (D)	By examining Table 2 for PCB in Soil it can be observed that an <i>Analytical Confidence Factor</i> of 0.8 is estimated to yield 96.1% negative results at a PCB concentration of 5 ppm Aroclor 1248 (0.5 Δ <i>Action Level</i> [10 ppm Aroclor 1248]). In this example it is judged that a 3.9% "false positive" rate would be acceptable for samples at 5 ppm. It is also noted that at 10 ppm (<i>Action Level</i>) the estimated rate of positive results is 88% while at 20 ppm (2.0 Δ <i>Action Level</i>) the incidence for false negative results is estimated to be <0.1%.
<i>Cutoff Concentration</i>	5 ppm as <u>Aroclor 1254</u> (E)	Performing the calculation A Δ B Δ C Δ D the result is 5.8 ppm. In this example the <i>Cutoff Concentration</i> used to classify the PCB assay results (after correction for dilution) was rounded down to 5 ppm to be conservative.

PENTACHLOROPHENOL RaPID ASSAY CHARACTERISTICS

Table 1 - Reactivity Factors for Pentachlorophenol RaPID Assay

Compound	<i>Reactivity Factor</i>
Pentachlorophenol	1.00
2,3,5,6-Tetrachlorophenol	0.54
2,3,4,6-Tetrachlorophenol	0.15

Table 2 - Analytical Confidence Factor Data

Pentachlorophenol in Water

<i>Analytical Confidence Factor</i>	@ 0.5 □ Action Level		@ 1.0 □ Action Level		@ 2.0 □ Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.4	0.6	26.6	73.4	<0.1	>99.9
0.8	97.0	3.0	10.6	89.4	<0.1	>99.9
0.7	89.4	10.6	3.0	97.0	<0.1	>99.9
0.6	73.4	26.6	0.6	99.4	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Pentachlorophenol in Soil

<i>Analytical Confidence Factor</i>	@ 0.5 □ Action Level		@ 1.0 □ Action Level		@ 2.0 □ Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	95.9	4.1	33.3	66.7	<0.1	>99.9
0.8	90.4	9.6	19.2	80.8	<0.1	>99.9
0.7	80.8	19.2	9.6	90.4	<0.1	>99.9
0.6	66.7	33.3	4.1	95.9	<0.1	>99.9
0.5	50.0	50.0	1.5	98.5	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 □ Action Level and false negative rates 0.1% or less at 2.0 □ Action Level. For example, applying a 0.8 *Analytical Confidence Factor (D)* (see worksheet on p. 4.5) for a Pentachlorophenol soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 90.4% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

PCB RaPID ASSAY CHARACTERISTICS

Table 1 - Reactivity Factors for PCB RaPID Assay

Compound	Reactivity Factor
Aroclor 1016	0.14
Aroclor 1232	0.19
Aroclor 1242	0.41
Aroclor 1248	0.85
Aroclor 1254	1.00
Aroclor 1260	1.56
Aroclor 1262	0.76
Aroclor 1268	0.16

Table 2 - Analytical Confidence Factor Data

PCB in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	20.3	79.8	<0.1	>99.9
0.8	99.4	0.6	4.8	95.2	<0.1	>99.9
0.7	95.2	4.8	0.6	99.4	<0.1	>99.9
0.6	79.8	20.3	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

PCB in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.1	0.9	27.8	72.2	<0.1	>99.9
0.8	96.1	3.9	12.0	88.0	<0.1	>99.9
0.7	88.0	12.0	3.9	96.1	<0.1	>99.9
0.6	72.2	27.8	0.9	99.1	<0.1	>99.9
0.5	50.0	50.0	0.2	99.8	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a PCB soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 96.1% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

TNT RaPID ASSAY CHARACTERISTICS

Table 1 - Reactivity Factors for TNT RaPID Assay

Compound	Reactivity Factor
TNT (2,4,6-Trinitrotoluene)	1.00
1,3,5-Trinitrobenzene	0.66

Table 2 - Analytical Confidence Factor Data

TNT in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	18.7	81.3	<0.1	>99.9
0.8	99.6	0.4	3.7	96.3	<0.1	>99.9
0.7	96.3	3.7	0.4	99.6	<0.1	>99.9
0.6	81.3	18.7	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

TNT in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	18.4	81.6	<0.1	>99.9
0.8	99.6	0.4	3.6	96.4	<0.1	>99.9
0.7	96.4	3.6	0.3	99.7	<0.1	>99.9
0.6	81.6	18.4	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a TNT soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 99.6% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

CYCLODIENES RaPID ASSAY CHARACTERISTICS**Table 1 - Reactivity Factors for Cyclodienes RaPID Assay**

Compound	Reactivity Factor
Dieldrin	1.00
Aldrin	1.25
Isodrin	1.04
Endrin	0.81
Heptachlor	0.62
Heptachlor-endo-epoxide	0.56
Chlordane	0.37
Endosulfan	0.22
Isobenzan	0.22
Toxaphene	0.09
Lindane	0.01

Table 2 - Analytical Confidence Factor Data

Cyclodienes in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	20.2	79.8	<0.1	>99.9
0.8	99.4	0.6	4.8	95.2	<0.1	>99.9
0.7	95.2	4.8	0.6	99.4	<0.1	>99.9
0.6	79.8	20.3	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Cyclodienes in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.9	0.1	22.4	77.6	<0.1	>99.9
0.8	98.8	1.2	6.4	93.6	<0.1	>99.9
0.7	93.6	6.4	1.2	98.8	<0.1	>99.9
0.6	77.6	22.4	0.1	99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a Cyclodienes soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 98.8% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

SECTION 5 - SELECTING THE RANGE (DILUTIONS)

GENERAL

SDI supplies RaPID Assay methods along with the appropriate reagents and equipment to prepare the sample for analysis. This system has been optimized to provide reliable results over a fixed range of concentrations that will often correspond to project needs. In addition, since the RaPID Assay methods can present results in quantitative (numerical) terms, the user has the flexibility to adjust the assay range to best suit a particular project. This can be accomplished by utilizing the range of results defined by the standard curve or by further diluting the sample into the range. This section provides a systematic approach to determining the recommended dilution for samples at a variety of concentrations. The resulting dilution will position the critical concentration at an optimum position in the assay standard curve. A worksheet for performing the calculations is provided to aid the user.

GENERAL PROCEDURE

DEFINITIONS:

Cutoff Concentration: the concentration of analyte that will be used to classify samples as positive or negative for an Action Level. This concentration should be expressed in the same units as the RaPID Assay *Standard 2 Concentration*. When following the procedure recommended below, the assay performance will be adjusted to provide greatest precision on samples at this concentration. See Section 4: Selecting a Cutoff Concentration for guidance.

Standard 2 Concentration: the concentration of analyte in the Standard 2 provided in the RaPID Assay kit. There are three standards provided in each RaPID Assay kit. The *Standard 2 Concentration* is in the most precise region of the standard curve. For purposes of these calculations, the *Standard 2 Concentration* should be expressed in the same units as the *Cutoff Concentration*. *Standard 2 Concentrations* for the RaPID Assay kits are

shown in Table 1 on the worksheet found at the end of this section.

Target Dilution Factor: the total dilution required to bring a soil or water sample at the *Cutoff Concentration* to a level at which the method is most precise.

Kit Sample Dilution: the dilution obtained by following the dilution scheme provided with the RaPID Assay method and associated sample preparation techniques. For example when analyzing soil samples according to the instructions provided with the PCB RaPID Assay, the RaPID Prep Soil Collection kit and the PCB RaPID Prep Sample kit, the total dilution of PCBs originally present in the soil sample is 2000. A complete list of *Kit Sample Dilutions* is provided in Table 1 on the worksheet found at the end of this section.

Working Sample the sample obtained after completing the sample preparation steps, if any, indicated in the SDI kit method. When no additional dilution is required, this is the sample to be added to the assay tube. In the case of soil samples, the *Working Sample* is the diluted soil extract. For water samples, it may be the water sample itself or a dilution of the sample, i.e., the 1:2 methanol dilution (1 part methanol plus 1 part sample) of the water in the PCB method.

PROTOCOL:

1. Determine the *Cutoff Concentration* for the sample(s) to be analyzed for the project.
2. Obtain the *Standard 2 Concentration* of the appropriate RaPID Assay kit from Table 1 on the worksheet.
3. Calculate the *Target Dilution Factor* by dividing the *Cutoff Concentration* by the *Standard 2 Concentration*.
4. Obtain the *Kit Sample Dilution* from Table 1 of the worksheet.
5. Calculate the ratio $[E]$ of the *Target Dilution* to the *Kit Sample Dilution* using the worksheet provided.

**SECTION 5
SELECTING THE RANGE**

6. Further dilute the *Working Sample* to the *Target Dilution* if necessary as recommended in the Dilution Procedure below.
7. Assay the diluted sample following the procedure provided in the appropriate RaPID Assay kit.
8. Multiply the results obtained by the *Target Dilution Factor* to obtain the concentration of analyte in its original matrix.
9. The calculated concentration of analyte (from Step 8) is compared to the *Cutoff Concentration* (from Step 1) and classified as positive or negative for the project Action Level.

DILUTION PROCEDURE

SDI provides a Universal Range Extension kit that includes materials and instructions for preparing dilutions in all ranges of interest for environmental analysis. The following procedure utilizes the materials in this kit.

DETERMINATION OF DILUTION FACTOR:

- If E is less than 0.5, it may be possible to use the dilution scheme provided in the SDI kit package inserts and a *Cutoff Concentration* as low as Standard 1. For further assistance, contact SDI Technical Services for recommendations.
- If E is between 0.5 and 3, perform no further dilutions, test the *Working Sample* directly in the RaPID Assay.
- If E is between 3 and 100, refer to the Dilution Chart that follows.
- If E is greater than 100, refer to the Dilution Chart that follows and select two smaller dilution factors whose product equals the desired larger dilution. For example: a dilution factor of 200X could be prepared by first diluting the extract by 20X followed by an additional serial dilution of 10X. Consult SDI Technical

Services if assistance is needed in making this determination.

MATERIALS:

RaPID Prep Universal Range Extension Kit (A00235)

RaPID Assay Sample Diluent (choose Sample Diluent for analyte of interest)

Adjustable Pipette (500 to 2500 μ L) (A00238)

*Precision pipettes and tips capable of delivering 100 and 250 μ L

*Required only if dilution is less than 25 or greater than 100.

PROTOCOL:

1. Write sample information on the labels provided in the kit. Apply labels to vials.
2. From **Column I** in Dilution Chart that follows the worksheet, find the *Dilution Factor (E)* from the worksheet that you have determined is appropriate for your project.
3. Determine the volume of Sample Diluent to pipette from **Column II**. Set the volume of Sample Diluent on the adjustable pipette. Attach a clean tip to the adjustable pipette. Transfer the appropriate volume of RaPID Sample Diluent into clean dilution vials. Refer to pipette instructions on setting volumes.
4. Using a clean tip and precision pipette, transfer the appropriate volume of *Working Sample* from **Column III** into each vial. Cap each vial and mix by inverting several times.
5. Continuing with Step 7 of the Protocol section of the General Procedure above, the mixture can now be measured as "sample" according to the package insert of the RaPID Assay kit. (Note: Additional dilution of the sample will, in general, decrease the effect of potential interferences reported in the method package insert.)

WORKSHEET

Cutoff Concentration _____ (A)

Standard 2 Concentration _____ (B)
(from Table 1)

Target Dilution Factor (A/B) _____ (C)

Kit Sample Dilution _____ (D)
(from Table 1)

Dilution Factor (C/D) _____ (E)

Volume of *Working Sample* _____
(from Column III of Dilution Chart, p. 5.4)

Volume of Sample Diluent _____
(from Column II of Dilution Chart)

Table 1 - SDI Kit Information

Analyte	Standard 2 Concentration (ppb)	Sample Matrix	Kit Sample Dilution
PCP	2 ppb	Water Soil	1 1000
PCB	1 ppb	Water Soil	2 2000
TNT	1.0 ppb	Water Soil	1 1000
Cyclodienes	7.5 ppb	Water Soil	1.33 100

Example -

To optimize the PCB RaPID Assay system for a 40 ppm in soil cutoff (Step numbers refer to the Protocol step numbers given earlier in the General Procedure section):

- 1) *Cutoff Concentration* _____ 40,000 ppb(A)
- 2) *Standard 2 Concentration* _____ 1 ppb (B)
- 3) *Target Dilution Factor (A/B)* _____ 40,000 (C)
- 4) *Kit Sample Dilution* _____ 2,000 (D)
- 5) *Dilution Factor (C/D)* _____ 20 (E)

From Dilution Chart (p. 5.4):

- Volume of *Working Sample* _____ 100 µL
- Volume of Sample Diluent _____ 1.9 mL
- 7) If the result on diluted sample from RaPID Assay is _____ 2.3 ppb (F)
 - 8) Then the concentration of sample in original matrix is (F × C) _____ 92,000 ppb(G)
 - 9) Compare (G) to *Cutoff Concentration* (A); greater is _____ Positive for
positive, less negative _____ Action Level

**SECTION 5
SELECTING THE RANGE**

Dilution Chart

I <i>Dilution Factor</i>	II Volume of Sample Diluent (µL)	III Volume of Working Sample (µL)
3	500	250
4	750	250
5	1000	250
6	1250	250
7	1500	250
8	1750	250
9	2000	250
10	2250	250
11	1000	100
12	1100	100
13	1200	100
14	1300	100
15	1400	100
16	1500	100
17	1600	100
18	1700	100
19	1800	100
20	1900	100
21	2000	100
22	2100	100
23	2200	100
24	2300	100
25	600	25
30	726	25
35	850	25
40	976	25
45	1100	25
50	1226	25
55	1350	25
60	1476	25
65	1600	25
70	1726	25
75	1850	25
80	1976	25
85	2100	25
90	2226	25
95	2350	25
100	2476	25
>100x	Combinations of the above dilutions	

Note: *Dilution Factors* from 25 to 100 are listed in increments of 5. The following formula can be used for this range of *Dilution Factors* to calculate Volume of Sample Diluent (Column II) to dispense for *Dilution Factors* (Column I) not listed in the chart. A 25 µL *Working Sample* is always used when this formula is applied:

$$\text{Volume of Sample Diluent (in } \mu\text{L)} = (25 \times \text{Dilution Factor}) - 25$$

SECTION 6- RPA-I™ ANALYZER

The RPA-I Analyzer is a laboratory benchtop-based, single wavelength, dual beam, microprocessor-controlled analyzer. It can read the absorbances of calibrators and samples, perform mathematical computations, and report raw absorbances and sample concentrations with statistics. For a complete and detailed description of the RPA-I, please refer to the *RPA-I RaPID Analyzer Operations Manual* (SDI Part No. A00046).

ENVIRONMENT

- 5° C to 33° C
- 10% to 85% humidity
- Flat, level surface away from strong sources of electromagnetic interference.
- No direct sunlight or drafts.
- Removed from sources of direct heat and moisture.
- Ventilation space at least 6 inches on sides and back.

UNPACKING AND INSTALLATION

1. Inspect the carton for visible signs of damage and note the condition of the SHOCK-WATCH indicator on the side of the carton. If damage has occurred, or a part is missing, immediately contact SDI.
2. Open the carton and remove the brown rectangular box from the grey packing material. (Save all boxes.) This box contains the power transformer, roll of paper, and

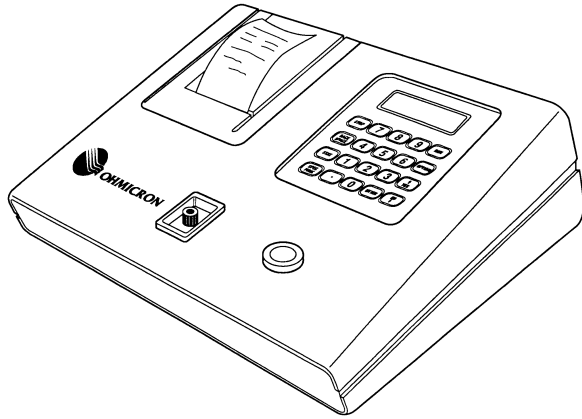
Program Cartridge. Refer to Figure 1 for identification of shipping carton contents.

3. Lift off the gray packing material to reveal the photometer. Remove it from the carton.
4. Insert the Program Cartridge (with the white label facing up) into the Program Cartridge Holder found on the rear panel of the instrument. Push in until the white label is no longer visible (Refer to Figure 2).
5. With the power **OFF** to the instrument, (bottom of the white toggle power switch should be depressed) insert the round end of the Power Transformer (notched end facing up) into the AC Power Connector found on the rear panel of the instrument. Plug the square end of the power cable into a grounded AC outlet.
6. The instrument is activated by depressing the top of the white toggle power switch. The instrument will perform a "Self Test." During this short test, the various electronic components of the RPA-I are automatically analyzed. This includes checks of EPROM and RAM memory. If there are any abnormalities in these areas, the RPA-I will alert the operator with an "ERROR" message. If all the parameters are satisfactory, the "Select Command" prompt will appear and the operator may continue.

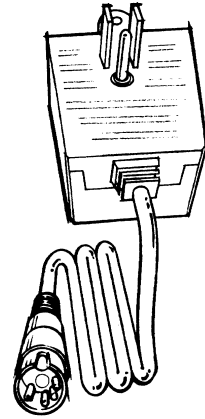
SHIPPING CARTON CONTENTS

The shipping carton should contain the following items:

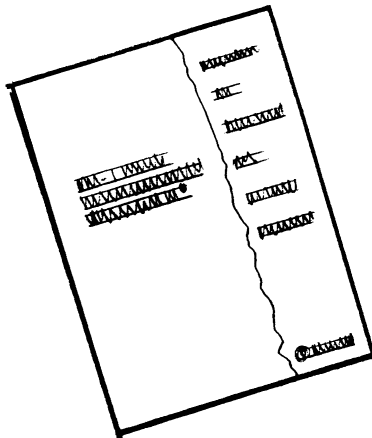
RPA-I Analyzer with a 450/600nm filter block.



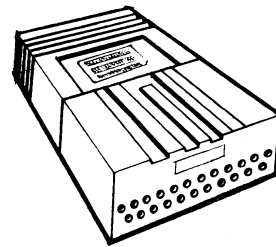
Domestic Power Cord/Mains Transformer



RPA-I Analyzer Operator's Manual



Program Cartridge



Printer Paper

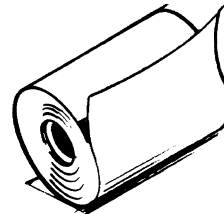


Figure 1. Shipping Carton Contents

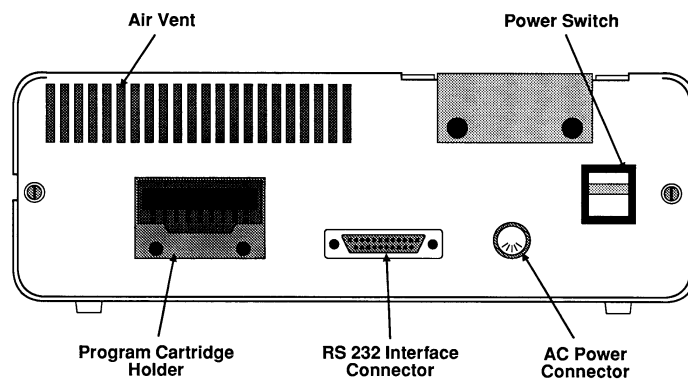


Figure 2. Rear Panel

SHORT OPERATING PROCEDURE FOR THE RPA-I



ALLOW THE RPA-I TO WARM UP FOR 30 MINUTES PRIOR TO USE. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter. The RPA-I performs a self test first. If all parameters are satisfactory, the "Select Command" prompt will appear. If there are abnormalities, an "Error" message will appear.

The RPA-I reports all results on a thermal paper printout. The unit is turned off by switching the power switch in the rear of the unit to the off position.

INSTRUMENT DISPLAYOPERATOR RESPONSE

SELECT COMMAND

Press RUN

RUN PROTOCOL: Aldicarb, Atrazine,
Alachlor, etc.Scroll using the YES [] or NO []
until the desired protocol appears.
Press ENTER.SPL. REPLICATES:
(1-5)Press 1 (Press 2 if analyzing samples in
duplicate, etc.). Press ENTER.BLANK TUBE
INSERT TUBEInsert tube with 1 mL of washing
solution/bufferEVALUATING TUBE
REMOVE TUBE (Beep)

Remove tube

CAL. #1 REP. #1
INSERT TUBEInsert first standard replicate (0 ppb
calibrator/tube #1).EVALUATING TUBE
REMOVE TUBE (Beep)

Remove Tube

Follow the prompts on the instrument display.

Note: Tube order is important here.
The RPA-I expects to see the
standards/calibrators in ascending order
in duplicate, starting with 0 ppb.

After all the standards (calibrators) have been evaluated, the instrument will display:

PRINTING DATA

Data will print.

LISTING XFORM
DATA

PRINTING CURVE

Curve will print only if programmed to
print (See Section 3 Special Functions -
Instrument Functions: Print Curve).CTRL. #1 REP. #1
INSERT TUBE

Insert Control Tube.

EVALUATING TUBE
REMOVE TUBE (Beep)

Remove Tube.

EDIT CALIBRATORS
YES/NOPress NO if it is not necessary to edit
the calibrators, press YES to edit (See
Section 3 Run).SPL. #1 REP. #1
INSERT TUBE

Insert first Sample Tube.

EVALUATING TUBE
REMOVE TUBE (Beep)

Remove Tube.

Follow the prompts on the instrument display. After all the samples have been evaluated, press STOP.

EXPLANATION OF DATA

Bolded areas are explained in the right hand column.

```

04-12-93 12:36:38

***** SDI *****

PROTOCOL :          PCB

TECH ID : _____
LOT #    : _____
EXP DATE: _____

Data Reduct:Lin.Reggression
Xformation:          Ln/LgtB
Read Mode  :          Absorbance
Wavelength :          450 nm
Units      :          PPB

EQUATION OF LINE :
-----
Slope      =          -0.690      □
Intercept  =          -0.253      □
Corr (r)   =          0.9977      □

Transformed Data :
-----
Conc          Abs
-----
-1.39       0.747      □
0.00       -0.334     □
1.61       -1.327     □

Calibrator Data:
-----
Conc          Abs          %CV          Predic
              Diff
              %Diff
-----
0.00          1.032
              1.024
Mean          1.028          0.4

0.25          0.689
              -0.002
              0.706
              -0.028
Mean          0.698          1.7
              -0.015
              -6.1

1.00          0.442
              0.042
              0.416
              0.212
Mean          0.429          4.2
              0.124
              12.4

5.00          0.228
              -0.730
              0.203
              0.280
Mean          0.216          8.4
              -0.265
              -5.3

```

Data Reduction

Method of transformation for data. Example, Ln refers to the natural log of the concentration and LgtB refers to the logit function of the absorbance divided by the absorbance at zero concentration.

Equation of Line

These values are the coefficients which describe a "best fit" or linear regression straight line where $\text{Logit}(B/B_0) = \text{slope} \times \text{Log}_e(\text{conc. in ppb}) + \text{intercept}$. The $\text{Corr}(r)$ is the correlation coefficient which indicates "goodness of fit" of the data to the best fit line. The square of this value represents the proportion of variance (on the y axis) that is explained by the linear regression.

Transformed Data

This section shows the average "transformed data" for each standard point. For example, $\text{Log}_e(0.25 \text{ ppb}) = -1.39$
 $\text{Logit}(\frac{0.698}{1.028} \text{ or } \frac{B}{B_0}) = 0.747$

Calibrator Data

0.689 = observed absorbance
0.25 = observed concentration
-0.002 = known conc.(0.25) - observed conc.(0.25)*
-0.8 = concentration diff (-0.002) ÷ observed conc. (0.25) x 100*
1.7 = standard deviation of observed absorbances ÷ mean (0.698) x 100
4.2 = coefficient of variation (%CV) is calculated using absorbances as above

*For accuracy, the data reduction software of the RPA-I utilizes seven significant digit numbers although only three are displayed or printed.

Control Data :			
Ctrl#	Abs	Conc	
1	0.272	3.05	
ID: _____			
Samples Data :			
Spl#	Abs	Conc	%CV
1	0.482	0.83	
	0.460	0.94	
Mean	0.471	0.89	8.8
ID: _____			
2	0.360	1.70	
	0.368	1.61	
Mean	0.364	1.66	3.8
ID: _____			
3	0.925	0.03nd	
	0.930	0.03nd	
Mean	0.928	0.03nd	5.7
ID: _____			
4	0.991	0.01nd	
	0.998	0.00nd	
Mean	0.995	0.01nd	22.1*
ID: _____			
5	0.927	0.03nd	
	0.921	0.03nd	
Mean	0.924	0.03nd	6.5
ID: _____			
6	0.230	4.20	
	0.233	4.10	
Mean	0.232	4.15	1.7
ID: _____			
7	1.038	nd	
	1.036	nd	
Mean	1.037	nd	
ID: _____			
END OF RUN			
04-12-93 12:39:24			

Control Data

Displays absorbance and concentration of control sample. This concentration should be compared to the reported range located on the control vial label to assure the quality of the run.

Sample Data

8.8 = this %CV is calculated using the sample concentrations

"nd" indicates concentration below the "Normal Range Low" value entered during the protocol setup. This value is the least detectable dose (LDD) for RaPID Assay protocols.

"*" indicates the %CV exceeds the parameter setting limit. This is common when the concentration is very low.

An "nd" without a concentration indicates the absorbance measured is greater than the absorbance of the 0 ppb standard therefore a concentration cannot be calculated.

OBSOLETE

SECTION 7 - RPA-III™ ANALYZER

The RPA-III RaPID Analyzer is a hand-held microprocessor-based unit with fully interchangeable filters for wavelength selection. The instrument features a liquid crystal display and is powered through a cable connected to an electrical outlet. By itself, the RPA-III does not provide a printout of results, nor does it perform mathematical functions. For a complete and detailed description of the RPA-III, please refer to the *RPA-III RaPID Analyzer Operations Manual* (SDI Part No. A00101).

Unpacking and Installation

1. Inspect the package for visible signs of damage and note the condition of the carton. If damage has occurred, or a part is missing, immediately contact SDI.
2. Open the carton and remove the photometer and power cord from the packing material.
3. Plug the square end of the power cord into a grounded 110 v AC outlet. Insert the other end into the back of the RPA-III.
4. The unit is turned off by unplugging the power cable from the unit.

SHORT OPERATING PROCEDURE FOR THE RPA-III

Before reading tubes, allow five minutes after powering the RPA-III for warm up. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter.

INSTRUMENT DISPLAY

STANDARDIZE? Y/N

ZERO BLANK

READ

00 0.000 ABS.

READ SAMPLE

01 X.XXX ABS.

02 X.XXX ABS.

OPERATOR RESPONSE

Press the **Zero/No** button.

Insert the blank tube containing at least 1 mL of Washing Solution/Buffer and press the **Zero/No** button.

The RPA-III reads the blank tube and zeros the instrument (NOTE: for optimum performance, re-zero the unit after every ten readings. To re-zero, insert the blank and press the **Zero/No** button twice.)

The unit displays the reading number and the absorbance. Remove tube.

Insert the first tube and press the **Read/Yes** button.

The tube is read and the absorbance is displayed. **RECORD THE ABSORBANCE VALUE.**

Repeat for all standards, control, and samples.

CALCULATIONS

Using the graph paper provided with the RaPID Assay Kit, draw the standard curve by plotting the B/B_0 ratios versus concentrations using the absorbance data obtained above. Graph papers are specific for each method. Use only the graph paper supplied with each kit.

The mean absorbance value for the 0 standard is the B_0 . The mean absorbance value for the other calibrators is the B value. Divide the absorbance of the standard, control or sample by the zero absorbance and multiply by 100 to obtain the % B/B_0 . Draw the best straight line through all standard % B/B_0 points (% B/B_0 - y-axis, concentration - x-axis). Using the % B/B_0 of the sample interpolate the concentration using the standard curve.

OBSOLETE

SECTION 8 - RPA-III™ FIELD KIT

The RPA-III RaPID Field Kit consists of the RPA-III Analyzer; a hand-held microprocessor-based unit with liquid crystal display, and a portable case which contains a rechargeable battery, printer and tube cover. The Field kit also contains a power cord for battery charging and optional AC operation. The RPA-III Field Kit is intended for use outside of the laboratory environment.

For a complete and detailed description of the RPA-III, please refer to the *RPA-III RaPID Analyzer Operations Manual* (SDI Part No. A00101).

UNPACKING AND INSTALLATION

1. Seat the RPA-III firmly in the case making sure the battery/printer connection is inserted into the photometer.
2. Assuming the battery is charged, turn on both the printer and the RPA-III via the two power toggle switches.

Refer to the RPA-III Operating Manual for complete and detailed descriptions of operations.

SHORT OPERATING PROCEDURE FOR THE RPA-III FIELD SYSTEM

Allow the RPA-III to warm up for five minutes after switching it on before reading tubes. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter.

INSTRUMENT DISPLAY

STANDARDIZE? Y/N

ZERO BLANK

READ

00 0.000 ABS.

READ SAMPLE

01 X.XXX ABS.

02 X.XXX ABS.

OPERATOR RESPONSE

Press the **Zero/No** button.

Insert the blank tube containing at least 1 mL of Washing Solution. Place the tube cover over the tube and seat it firmly on the reader. Press the **Zero/No** button.

The RPA-III reads the blank tube and zeros the instrument (NOTE: for optimum performance, re-zero the unit after every ten readings. To re-zero, insert the blank and press the **Zero/No** button twice.)

The unit displays the reading number and the absorbance. Remove tube.

Insert the first tube. Place the tube cover over the tube. Press the **Read/Yes** button. The absorbance is displayed and printed.

The tube is read and the absorbance is displayed. **RECORD THE ABSORBANCE VALUE.**

Insert the next tube. Place the tube cover over the tube and press the **Read/Yes** button. The absorbance is displayed and printed.

CALCULATIONS

Refer to the appropriate *RPA-III Field Protocol* found in the *RPA-III RaPID Analyzer Operations*

Manual for directions on *Calculations and Interpretation of Results* for qualitative or semi-quantitative results.

SECTION 9 - RPA-VI™ ANALYZER

The RPA-VI Analyzer is a hand-held, microprocessor-controlled unit for absorbance measurements at 450 nm. The instrument features a liquid crystal display (LCD) and is powered by a standard 9 Volt battery or from an AC/DC adapter. For a detailed description of the RPA-VI and its functions, refer to the *RPA-VI RaPID Photometric Analyzer Operator's Manual* (SDI Part No. A00249).

UNPACKING AND INSTALLATION

1. Inspect the package for visible signs of damage and note the condition of the carton. If damage

has occurred to the RPA-VI or if a part is missing, contact SDI immediately.

2. Open the carton and remove the analyzer. If a printer was purchased, connect the RPA-VI and printer with the serial cable. Set the printer **ON-1-2** switch to **1**.
3. Press the **On/Blank/Off** button. The LCD should show **SDI v. x.x** followed by **Blank needed**. The printer should print **SDI v. x.x** followed by **Absorbance**.

SHORT OPERATING PROCEDURE FOR THE RPA-VI

Before reading tubes, allow the RPA-VI about one minute for warm-up, after which the LCD should read **BLANK NEEDED**. Avoid analyzing tubes with air bubbles, foam, scratches, or foreign matter.

INSTRUMENT DISPLAY

BLANK NEEDED

BLANK 0.00 OK

ABS IS 0.000

ABS IS 0.2xx

ABS IS X.XXX

OPERATOR RESPONSE

Insert the blank tube containing at least 1 mL of Washing Solution/Buffer

The RPA-VI automatically zeros itself. Press the **On/Blank/Off** button to store the blank reading.

Leave in the blank and confirm the zero reading.

Remove the blank tube. The empty chamber reading should be between 0.2 and 0.3 OD.

Insert the first tube; wait approximately 2-3 seconds for the reading to settle, then **RECORD THE ABSORBANCE VALUE**. Repeat for standards, controls, and samples. If the printer is connected, press the **Print** button to record the value.

CALCULATIONS

The mean absorbance for the Zero Standard is B_0 and the mean absorbance for the other calibrators and samples is B . Divide the absorbance of standards, controls, and samples by B_0 and multiply by 100% to obtain $\%B/B_0$. Using the graph paper provided with each RaPID Assay Kit, draw the standard curve by plotting $\%B/B_0$ (y-axis) versus calibrator

concentrations (x-axis). The graph paper is specific to a given method; use only the graph paper supplied with the kit. Draw the best-fit straight line through the plotted points and obtain the sample concentration from the x-axis for a given $\%B/B_0$.

SECTION 10 - OTHER SPECTROPHOTOMETERS

REQUIREMENTS

This section describes the requirements for use of RaPID Assays with tube readers other than the RPA series photometers. These requirements are:

Absorbance Range: 0 - 2.0 AU

Wavelength: 450 nm.

Detection volume: 1 mL in cuvettes.

Drift: \leq 0.005 AU per hour at 0 AU.

Linearity: \leq 0.005 A or 2% difference from calculated regression line. Correlation coefficient, $r = 0.9995$ or better.

Results reporting: to a display or printer.

Sipper cell: the operator must first establish pump cycle times that allow for 1 mL to be drawn up from the tube and provide delivery to the flow cell to be read. Once this has been established, a return cycle

of two times the pick-up volume should be returned to avoid cross-over contamination.

Throughput: ability to read the tubes within *15 minutes* after the addition of stop solution to the assay tube.

CALCULATIONS

Using the graph paper provided with the RaPID Assay Kit, draw the standard curve by plotting the B/B_0 ratios versus concentrations using the absorbance data obtained above. Graph papers are specific for each method. Use only the graph paper supplied with each kit.

The mean absorbance value for the 0 standard is the B_0 . The mean absorbance value for the other calibrators is the B value. Divide the absorbance of the standard, control or sample by the zero absorbance and multiply by 100 to obtain the % B/B_0 . Draw the best straight line through all standard % B/B_0 points (% B/B_0 - y-axis, concentration - x-axis). Using the % B/B_0 of the sample interpolate the concentration using the standard curve.

SECTION 11 - FIXED VOLUME PIPETTES

ASSEMBLY

Secure a tip on the pipette nose by pressing the nose cone **firmly** into a tip contained in the pipette tip rack.



USE

GENERAL:

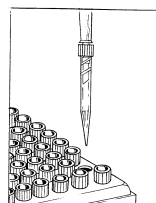
The piston stroke is divided into three sections: measuring, blow-out, and tip ejection.

First stop: required volume is defined and dispensed

Second stop: dispense any residual liquid

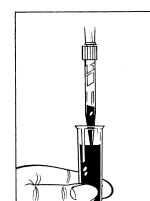
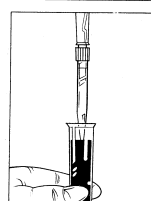
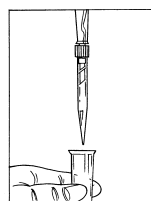
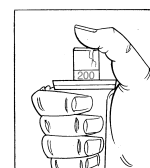
Third stop: tip ejection

Practice several pipette transfers with water using the following procedure:



FILLING:

1. Keep pipette almost vertical.
2. Press button down to the first stop. Immerse tip 2-3 mm into the liquid.
3. **Slowly** allow the button to glide back (never let it snap back). Avoid drawing air bubbles.
4. Slide tip out along the inside of the vessel.



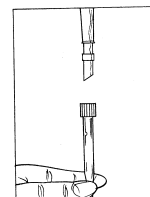
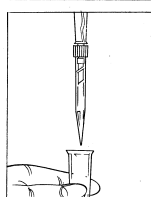
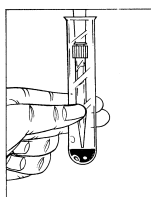
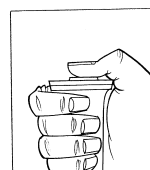
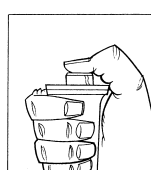
1

2

3&4

DISPENSING:

5. Insert pipette almost to the bottom of the test tube. Touch the tip to the side of the test tube about 5 mm above the dispensed liquid. Press button down to the **second stop**.
6. Remove pipette from the test tube.
7. Press button down to the third stop and discard the tip.



5

6

7

IMPORTANT: A new tip should be used for each standard or sample.

SECTION 12 - TRI-VOLUME PIPETTES

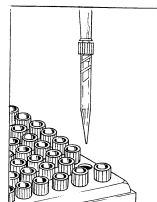
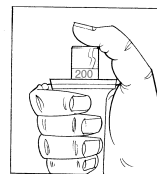
ASSEMBLY

Secure a tip on the pipette nose by pressing the nose cone **firmly** into a tip contained in the pipette tip rack.

USE

The tri-volume pipette will deliver the volume displayed opposite the red mark.

To select the volume to be delivered, press the button down to the horizontal mark (middle of the numbers) in the volume ring (e.g. 100--200--250) and turn to the right or left. The button must be returned to the fully extended position (numbers visible) to lock the required volume into place.



GENERAL:

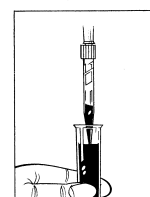
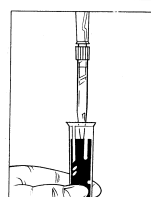
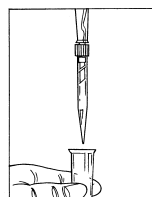
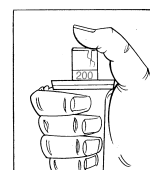
The piston stroke is divided into three sections: measuring, blow-out, and tip ejection.

First stop: required volume is defined and dispensed.

Second stop: dispense any residual liquid.

Third stop: tip ejection

Practice several pipette transfers with water using the following procedure:



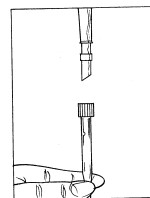
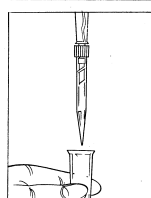
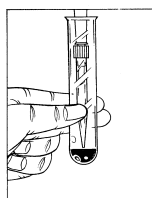
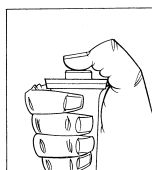
1

2

3&4

FILLING:

1. Keep pipette almost vertical.
2. Press button down to the first stop. Immerse tip 2-3 mm into the liquid.
3. **Slowly** allow the button to glide back (never let it snap back). Avoid drawing air bubbles.
4. Slide tip out along the inside of the vessel.



5

6

7

DISPENSING:

5. Insert pipette almost to the bottom of the test tube. Press button down to the **second stop**.
6. Remove pipette from the test tube.
7. Press button down to the third stop and discard the tip.

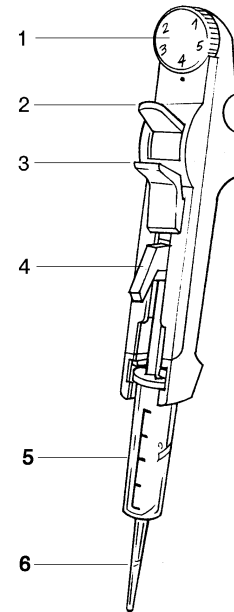
IMPORTANT: A new tip should be used for each standard or sample.

SECTION 13 - REPEATER PIPETTES

ASSEMBLY

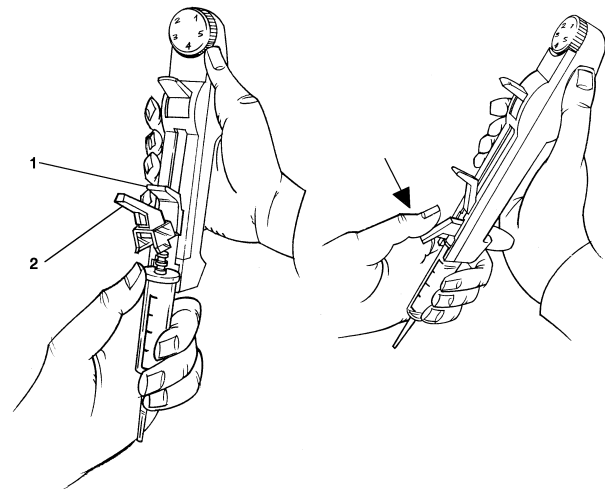
DESIGN PRINCIPLE:

1. Volume Selection Dial: To determine the pipette volume, the dial setting (1-5) is multiplied by the minimum pipette volume of the reservoir. See example under Reservoir/Use of the Repeater Pipette.
2. Pipette Lever: To deliver the selected volume, press this lever down until it stops.
3. Filling Lever: To fill the reservoir, slide this lever upward.
4. Locking Clamp: The locking clamp serves to firmly clamp the reservoir into the pipette.
5. Reservoir
6. Reservoir Cone



INSERTING A RESERVOIR:

1. Slide the filling lever (1) down until it stops.
2. Raise the locking clamp (2) upward.
3. Insert the reservoir until it clicks into position. Be sure the reservoir plunger is fully inserted into the barrel and the filling lever is completely down before attaching the reservoir to the pipette.
4. Lower the locking clamp to secure the reservoir in place.



SECTION 14 - MAGNETIC RACK

USE

The magnetic rack is composed of two parts: the top rack that firmly holds the test tubes in place and the bottom base which contains the magnets.

ASSEMBLY

Place the rack on top of the base making sure they fit together securely and the test tubes are pushed down as far as they will go into the base.

USE OF THE ASSEMBLED RACK

For separation steps (washing and decanting) - to pull the magnetic particles to the sides of the tubes allowing the unbound components to be discarded.

DISASSEMBLY

Separate the top rack from the bottom base.

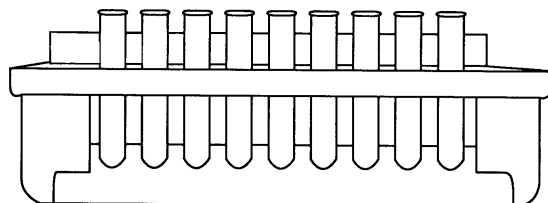
USE OF THE DISASSEMBLED RACK

For incubation steps - to allow the magnetic particles to remain suspended throughout the solution.

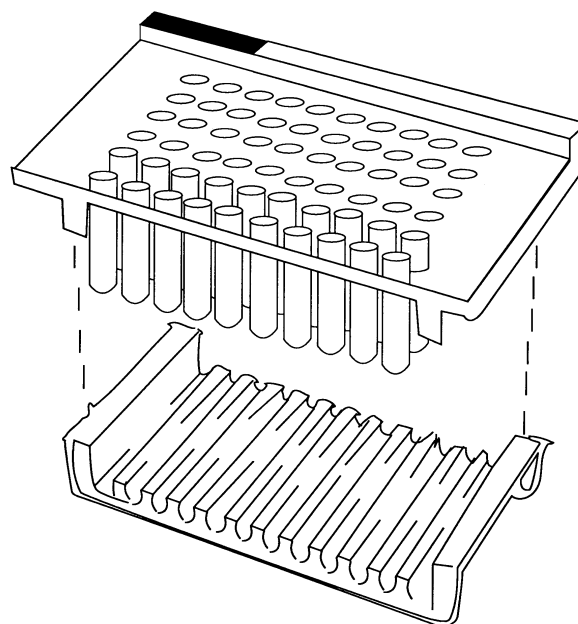
CARE

Do not submerge base in water. Clean with warm water and mild detergents.

CAUTION: Exposure to extreme heat or organic solvents could effect product's performance. Do not under any circumstances attempt to disassemble base to observe magnets. Magnets will be violently attracted to each other.



Assembled View



Disassembled View

OBSOLETE

SECTION 15 - SOIL EXTRACTION

GENERAL

The RaPID Assay kits are combined with the RaPID Prep[®] Soil Collection Kit and the appropriate Sample Extraction Kits to provide an integrated system for soil extraction and analysis. The system allows for reliable, convenient and cost effective determinations at the field testing or remediation site.

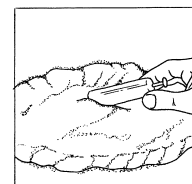
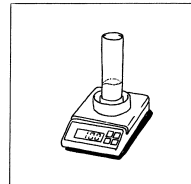
Before performing your first analysis, read entire package insert found in each box.

REQUIRED MATERIALS

- RaPID Prep Soil Collection Kit
- Appropriate RaPID Prep Sample Extraction Kit
- Stopwatch or clock with second hand
- Permanent marking pen
- Protective gloves
- Digital balance (optional)

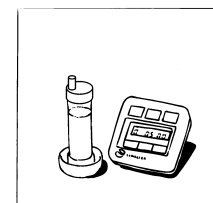
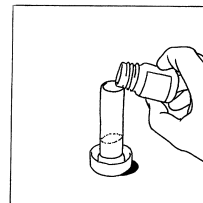
SAMPLING

Using the Soil Sample Collection Device from the Soil Collection Kit, collect 10 g of soil by weight or volume as directed in the package insert.



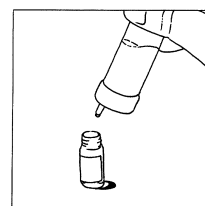
EXTRACTION

1. Add entire contents of the Extraction Solution to the Collection Device.
2. Replace screw cap.
3. Process by carefully following the package insert.
4. Let stand for 5 minutes.



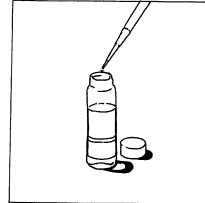
FILTRATION

1. Remove screw cap and attach Filter Cap.
2. Attach plunger rod to device.
3. Remove luer cap and filter extract into Extract Collection Vial.



DILUTION

1. Add the required volume of filtered extract to a vial of Extract Diluent using the pipette provided.
2. Cap and mix by inverting several times.
3. Refer to section in this guide on *Selecting the Range* for further instructions on additional dilutions that may be required and determination of the dilution factor.



ASSAY

Analyze the diluted extract as "sample" according to the appropriate RaPID Assay procedure.

Multiply the results of the extracted soil samples by the appropriate dilution factor

USE

RESERVOIR:

The minimum pipetting volume and the maximum filling capacity are shown on each reservoir. The volume to be pipetted is obtained by multiplying the set number located on the volume selection dial by the minimum pipetting volume of the reservoir.

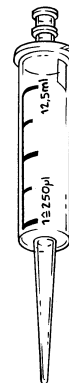
Example: 12.5 mL Reservoir:

Maximum fill volume = 12.5 mL

Minimum pipetting volume, Dial set to 1 = 250 μ L

At dial setting 4: $4 \times 250 \mu\text{L} = 1000 \mu\text{L}$

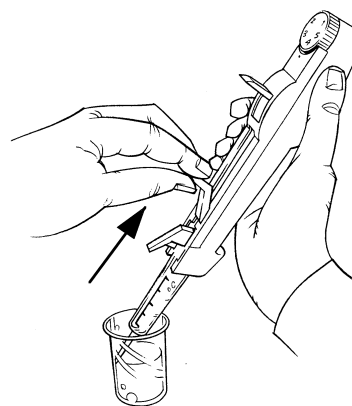
Pipetted volume: 1000 μ L



Practice several pipette transfers with water using the following procedure:

FILLING:

1. Immerse the reservoir cone into the liquid.
2. Fill by **slowly** sliding the filling lever upward.
3. Discard the first pipetting step by completely depressing the pipetting lever with a smooth, continuous motion until it stops.
4. Allow the pipetting lever to return to its starting position.



The repeater pipette is now ready for operation.

ADDITIONAL FILLING PRECAUTIONS:

Sliding the filling lever too quickly can cause excessive vacuum. This can cause tiny air bubbles to accumulate in the liquid which may lead to pipetting inaccuracies. If this occurs, empty and refill the reservoir.

It is important to discard the first pipetting step in order to release any residual pressure from the pipetting system after filling and to prepare the system for precise pipetting.

Small bubbles in the reservoir beneath the piston do not affect pipetting accuracy because an incorporated residual stroke lock prevents the pipetting of any residual material after the last dispensing.

It is not necessary to completely fill the reservoir; partially filling reservoirs does not affect pipetting accuracy.

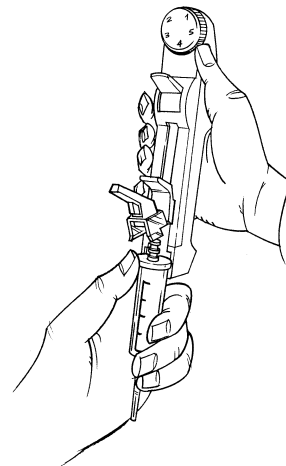
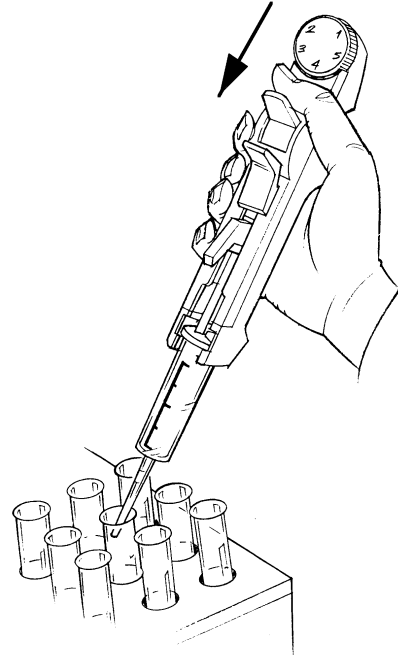
PIPETTING:

A set of five labeled 12.5 mL (1=250 μ L) reservoirs is needed for each RaPID Assay. They should be labeled with the assay name and the following: "conjugate 250 μ L," "particles 500 μ L," "wash 1 mL," "color 500 μ L," and "stop 500 μ L."

1. Check the volume selection dial for the appropriate setting.
 - Enzyme conjugate Ⓢ set dial at 1
 - Magnetic particles Ⓢ set dial at 2
 - Wash solution Ⓢ set dial at 4
 - Color reagent Ⓢ set dial at 2
 - Stop reagent Ⓢ set dial at 2
2. Hold the repeater at an angle so the tip is about one half inch below the tube rim without touching the rim or tube wall with the reservoir cone and aim the cone to deliver the liquid down the inside wall of the test tube.
3. Dispense the liquid by completely depressing the pipetting lever with a smooth, continuous motion until it stops.
4. Allow the pipetting lever to return to its starting position and repeat delivery into the next tube.
5. After the pipetting is completed, return the unused reagent to its container by holding the repeater unit over the container and pressing the filling lever down until it stops.
6. Prior to storage of the reservoirs, rinse the dedicated reservoirs twice with distilled water (keeping the reservoir in the pipette) by filling with 12.5 mL of distilled water each time and discarding the contents into a sink. Store syringes assembled (plunger inserted into barrel). Keep washed assembled syringes separated from each other. (Hint: An empty tray from the fixed volume pipette tips makes a handy storage rack). Reservoirs should be changed periodically (after 5-10 uses) since precision deteriorates with use.

REMOVING THE RESERVOIR:

Once the filling lever (1) is completely down, raise the locking clamp (2) upward and remove the reservoir.



SECTION 16 - START-UP

**MATERIALS NECESSARY TO
PERFORM A RAPID ASSAY**

1. RaPID Assay test kit with assay protocol, flowchart and package insert.
2. RPA-I, RPA-III with operating instructions (or equivalent spectrophotometer capable of reading 450 nm in a 1 mL sample size).
3. Magnetic rack separation unit.
4. Precision pipettes and appropriate tips, capable of delivering 100 μ L, 200 μ L, or 250 μ L depending on method.
5. Repeating pipette, with appropriate reservoirs, capable of delivering 250 μ L, 500 μ L, and 1000 μ L.
6. Thermolyne Maxi Mix™, Vortex Genie™ (or equivalent vortex mixer).
7. Disposable serological 5 or 10 mL glass or plastic pipettes for dispensing equal amounts of Chromogen and Hydrogen Peroxide to form the color reagent.
8. Pipette filler/bulb (for use with above disposable pipettes).
9. 20 mL (or larger) glass or plastic beaker for preparation of color reagent. Be sure the beaker has been thoroughly cleaned and rinsed. If contaminants are present, the reagent will develop a blue color and should be discarded.
10. Blotting paper for decanting steps (usually use a stack of 5 kitchen type paper towels).
11. Permanent marking pens for labeling tubes.
12. Instructional video (optional).
13. Laboratory timer or wrist watch.

ASSAY TIPS

Gently blot. Avoid **banging** the rack during the decanting steps.

Decant with the magnetic base joined to the top rack.

Wait a full two minutes for each separation step.

Bring samples and reagents fully to room temperature.

Vortex when specified without foaming.

Pay attention to the number of washes (2) and the amount of wash solution added (1 mL).

Add color reagent without the magnetic base joined to the top rack.

Remove as much liquid as possible from the test tubes by slowly decanting. A few droplets will remain in the tube.

Assure precise pipetting techniques.

Swirl the magnetic particles, without foaming, prior to addition.

PREPARATION AND RUNNING

Prior to performing your first RaPID Assay please do the following:

1. Read the entire package insert (found inside the box of reagents).
2. View the videotape (if available).
3. Gather together all the materials necessary to perform a RaPID Assay in a work space.
4. Remove the RaPID Assay kits from the refrigerator.
5. Remove the bottles from the kit box and place them on a countertop.
6. Allow the reagent bottles to come to room temperature.
7. Turn on the RPA-I or other spectrophotometer. The RPA-I should be warmed-up for 30 minutes.
8. Practice using the fixed volume and repeater pipettes with distilled water.
9. Label the top portion of the test tubes, with a permanent marker, in the following manner starting with the standard curve (in duplicate) followed by a control tube and sample tubes (in singlet).

<u>Tube#</u>	<u>Contents of Tube</u>
1,2	Zero Standard
3,4	Standard 1
5,6	Standard 2
7,8	Standard 3
9	Control
10	Sample #1
11	Sample #2
12	Etc.

10. Label 5 - 12.5 mL (1 = 250ml) reservoirs, with a permanent marker, with the following:
 - the first reservoir = conjugate 250ml
 - the second reservoir = particles 500ml
 - the third reservoir = wash 1ml
 - the fourth reservoir = color 500ml
 - the fifth reservoir = stop 500ml

In addition, add the name of the pesticide you are testing for to each syringe.

11. Continue with the **Assay Procedure** section of the package insert or flowchart.

Expected Results for RaPID Assays

- %CVs between standard duplicates of 10% or less.
- Absorbance readings for the 0 ppb standard greater than or equal to 0.800 for all assays.
- Corr (r) of 0.990 or greater for all assays.

SECTION 17 - ASSAY WORKSHEET

PURPOSE OF WORKSHEET

To aid in your evaluation of the assay kit, this familiarization run (a typical calibration curve plus proficiency samples) should be performed. Operators should repeat this run until the %CV of absorbances are consistently better than 10%. Experienced operators will obtain %CV's approaching 5% or better. The concentration values obtained on the control and the proficiency samples should be within the stated ranges.

FAMILIARIZATION RUN

Include in the run each standard in duplicate, a single control, and each proficiency sample in duplicate. Following the instructions in the kit package insert and found elsewhere in this manual, perform the assay.

RESULTS

RPA-I:

1. Observe the absorbance, the absorbance %CV, and the predicted result for each standard from the RPA-I printout (see Section 1 for a description of the printout).
2. Calculate the mean, SD, and %CV for the results from the standards and proficiency samples using a statistical calculator or the formulas given below the worksheet. Record in the following worksheet. Note that the %CV on the results may be significantly different than the absorbance %CV.

3. Compare to the above guidelines.

RPA-VI OR OTHER PHOTOMETERS:

1. Record the absorbance value for each standard or sample in the worksheet which follows.
2. Calculate the mean, standard deviation (SD), and %CV on the absorbances for each standard and sample using a statistical calculator or the formulas given below the worksheet.
3. Using the graph paper provided with the kit, prepare a standard curve as described in the "Results" section of the kit package insert under "Manual Calculations". Read all samples and calibrators as individual points from the standard curve. Use the worksheet which follows to record the results.
4. Calculate and record the mean, SD, and %CV for the results using a statistical calculator or the formulas given below the worksheet.
5. Compare to the above guidelines.

INTERPRETATION

If you run two curves and do not achieve acceptable %CV's on the absorbances call SDI Technical Support (1-800-544-8881) to discuss your results.

**SECTION 17
ASSAY WORKSHEET**

NAME: _____

DATE: _____

METHOD: _____

LOT NUMBER: _____

EXPIRATION DATE: _____

Tube #	Contents	Absorbance	%CV of Absorbance	Result [ppb]	% CV of Result
1	0 ppb Standard				
2	0 ppb Standard				
3	Standard #1				
4	Standard #1				
5	Standard #2				
6	Standard #2				
7	Standard #3				
8	Standard #3				
9	Control				
10	Sample A				
11	Sample A				
12	Sample B				
13	Sample B				
14	Sample C				
15	Sample C				

Calculations -

n = # of samples Mean: $\bar{x} = \Sigma x/n$

Standard Deviation (S.D.): = $\sqrt{(\Sigma(x - \bar{x})^2)/(n - 1)}$

Percent Coefficient of Variance (%CV): = $(S.D./ \bar{x}) \times 100$

SECTION 18 - TROUBLESHOOTING

Symptom	Cause	Corrective Action
Increased Absorbance	<p>Long incubation.</p> <p>Washed tubes only once.</p> <p>Washed tubes with less than 1 mL of Washing Solution.</p> <p>Warm reagents.</p>	<p>Adhere to incubation times.</p> <p>Be sure to wash tubes twice.</p> <p>Check pipette for delivery of 1 mL of Washing Solution.</p> <p>Bring reagents to room temperature.</p>
Decreased Absorbance	<p>Banging rack during decanting.</p> <p>Decanting without top rack joined to magnetic base.</p> <p>Short incubation.</p> <p>Did not wait 2 minutes between washings.</p> <p>Cold reagents.</p> <p>Did not vortex after addition of color reagent or particles.</p> <p>Addition of color reagent while top rack is joined to magnetic base.</p>	<p>Gently blot tubes, don't bang.</p> <p>Join rack and base prior to decanting.</p> <p>Adhere to incubation times.</p> <p>Allow particles to separate between washings.</p> <p>Bring reagents to room temperature.</p> <p>Vortex tubes after the addition of color reagent and particles.</p> <p>Separate rack from base before adding color reagent.</p>
Higher than expected results	<p>Presence of cross reactants, particulate matter, or other interferences in the sample.</p> <p>Inaccurate standard curve.</p> <p>Expired reagents and/or kit.</p> <p>Drift in sample results from beginning to end of run.</p> <p>Sample dilution inadequately mixed.</p>	<p>For particulate matter, filter samples with a 0.2 μm filter and re-assay. For cross reactants and possible interfering substances, dilute sample and re-assay.</p> <p>Re-run standard curve.</p> <p>Discard and replace with a fresh kit.</p> <p>Add all reagents in a consistent manner to entire rack within one minute.</p> <p>Be sure to thoroughly mix the diluted extract by inverting several times.</p>

**SECTION 18
TROUBLESHOOTING**

Symptom	Cause	Corrective Action
Lower than expected results	<p>Standards contaminated with analyte.</p> <p>Inaccurate standard curve.</p> <p>Sample dilution inadequately mixed.</p> <p>Cold Extraction Solution.</p> <p>Extraction of extremely wet sample (>40% moisture content).</p> <p>Inadequate agitation during extraction step.</p> <p>Insufficient time for extraction step.</p> <p>Inaccurate dilution of sample extract.</p> <p>Expired kit.</p>	<p>Discard and replace with a fresh kit.</p> <p>Re-run standard curve.</p> <p>Be sure to thoroughly mix the diluted extract by inverting several times.</p> <p>Repeat extraction. Cold Extraction Solution (4°C) can lower extraction efficiency 25-50% compared to 25°C.</p> <p>Air dry sample or correct for moisture content.</p> <p>Repeat extraction. Shake with sufficient force to break-up clumps.</p> <p>Carefully observe extraction times as described in the package insert. Extending the extraction time will increase the extraction efficiency.</p> <p>Repeat dilution using appropriate volume. Pipetting of sample extract should be performed quickly to avoid loss of sample.</p> <p>Discard and replace with a fresh kit.</p>
Increased % CV's	<p>Banging rack during decanting.</p> <p>Decanting without top rack joined to magnetic base.</p> <p>Did not vortex after addition of color reagent or particles.</p> <p>Addition of 2 mL of wash solution instead of 1 mL.</p> <p>Forgot to wait 2 minutes for separation.</p>	<p>Blot, don't bang.</p> <p>Join rack and base prior to decanting.</p> <p>Vortex tubes after the addition of color reagent and particles.</p> <p>Check pipette for delivery of 1 mL of liquid.</p> <p>Allow particles to separate between washings.</p>

Symptom	Cause	Corrective Action
Increased % CV's (cont.)	<p>Excessive wash solution remaining in tubes after decanting (may appear as bubbles).</p> <p>Imprecise addition of reagents.</p> <p>Neglecting to vortex during the wash steps in the PCB procedure.</p>	<p>After decanting, while holding top rack and bottom base together, allow tubes to drain in an inverted position for a few minutes. Also obtain more absorbent toweling.</p> <p>Replace reservoir. Pipettes may need maintenance.</p> <p>In the PCB RaPID Assay after adding the Washing Solution to each tube, vortex each tube for 1-2 seconds.</p>

SECTION 19 - QUICK GUIDE

GENERAL

This section contains materials that are commonly used by experienced users. Prior to referring to this section, follow the general instructions found in Section 1 - Introduction for use of this manual. This section includes the following:

- Worksheet - Selecting a Cutoff Concentration
- Pentachlorophenol RaPID Assay Characteristics
- PCB RaPID Assay Characteristics
- TNT RaPID Assay Characteristics
- Cyclodienes RaPID Assay Characteristics
- Worksheet - Selecting the Range (Dilutions)
- Short Operating Procedure for the RPA-I

WORKSHEET

Required Action Level _____ (A)

Reactivity Factor _____ (B)
(from Table 1)

Extraction Recovery Factor _____ (C)
(see p. 4.2)

Analytical Confidence Factor _____ (D)
(from Table 2)

Cutoff Concentration _____ (E)
A Δ B Δ C Δ D

Example -

To optimize the PCB RaPID Assay system for detection Aroclor 1248 at 10 ppm in soil:

<i>Required Action Level</i>	10 ppm as <u>Aroclor 1248</u> (A)	An <i>Action Level</i> of 10 ppm of Aroclor 1248 is assumed for this example. See <i>Action Level</i> in text above.
<i>Reactivity Factor</i> (from Table 1)	<u>0.85</u> (B)	Since the PCB to be detected in this example is Aroclor 1248, the <i>Reactivity Factor</i> obtained from Table 1 of the PCB RaPID Assay Performance Characteristics is 0.85. Using this factor allows Aroclor 1254 calibrators to be used in assessment of the cutoff concentration.
<i>Extraction Recovery Factor</i>	<u>0.85</u> (C)	The <i>Extraction Recovery Factor</i> of 0.85 is obtained from the RaPID Prep Sample Extraction kit package insert. Preferably, this factor would be obtained from a spiked matrix determination.
<i>Analytical Confidence Factor</i> (from Table 2)	<u>0.80</u> (D)	By examining Table 2 for PCB in Soil it can be observed that an <i>Analytical Confidence Factor</i> of 0.8 is estimated to yield 96.1% negative results at a PCB concentration of 5 ppm Aroclor 1248 (0.5 Δ <i>Action Level</i> [10 ppm Aroclor 1248]). In this example it is judged that a 3.9% "false positive" rate would be acceptable for samples at 5 ppm. It is also noted that at 10 ppm (<i>Action Level</i>) the estimated rate of positive results is 88% while at 20 ppm (2.0 Δ <i>Action Level</i>) the incidence for false negative results is estimated to be <0.1%.
<i>Cutoff Concentration</i>	5 ppm as <u>Aroclor 1254</u> (E)	Performing the calculation A Δ B Δ C Δ D the result is 5.8 ppm. In this example the <i>Cutoff Concentration</i> used to classify the PCB assay results (after correction for dilution) was rounded down to 5 ppm to be conservative.

PENTACHLOROPHENOL RaPID ASSAY CHARACTERISTICS**Table 1 - Reactivity Factors for Pentachlorophenol RaPID Assay**

Compound	Reactivity Factor
Pentachlorophenol	1.00
2,3,5,6-Tetrachlorophenol	0.54
2,3,4,6-Tetrachlorophenol	0.15

Table 2 - Analytical Confidence Factor Data

Pentachlorophenol in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.4	0.6	26.6	73.4	<0.1	>99.9
0.8	97.0	3.0	10.6	89.4	<0.1	>99.9
0.7	89.4	10.6	3.0	97.0	<0.1	>99.9
0.6	73.4	26.6	0.6	99.4	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Pentachlorophenol in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	95.9	4.1	33.3	66.7	<0.1	>99.9
0.8	90.4	9.6	19.2	80.8	<0.1	>99.9
0.7	80.8	19.2	9.6	90.4	<0.1	>99.9
0.6	66.7	33.3	4.1	95.9	<0.1	>99.9
0.5	50.0	50.0	1.5	98.5	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a Pentachlorophenol soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 90.4% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

PCB RaPID ASSAY CHARACTERISTICS

Table 1 - Reactivity Factors for PCB RaPID Assay

Compound	Reactivity Factor
Aroclor 1016	0.14
Aroclor 1232	0.19
Aroclor 1242	0.41
Aroclor 1248	0.85
Aroclor 1254	1.00
Aroclor 1260	1.56
Aroclor 1262	0.76
Aroclor 1268	0.16

Table 2 - Analytical Confidence Factor Data

PCB in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	20.3	79.8	<0.1	>99.9
0.8	99.4	0.6	4.8	95.2	<0.1	>99.9
0.7	95.2	4.8	0.6	99.4	<0.1	>99.9
0.6	79.8	20.3	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

PCB in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.1	0.9	27.8	72.2	<0.1	>99.9
0.8	96.1	3.9	12.0	88.0	<0.1	>99.9
0.7	88.0	12.0	3.9	96.1	<0.1	>99.9
0.6	72.2	27.8	0.9	99.1	<0.1	>99.9
0.5	50.0	50.0	0.2	99.8	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a PCB soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 96.1% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

TNT RaPID ASSAY CHARACTERISTICS**Table 1 - Reactivity Factors for TNT RaPID Assay**

Compound	Reactivity Factor
TNT (2,4,6-Trinitrotoluene)	1.00
1,3,5-Trinitrobenzene	0.66

Table 2 - Analytical Confidence Factor Data

TNT in Water

Analytical Confidence Factor	@ 0.5 □ Action Level		@ 1.0 □ Action Level		@ 2.0 □ Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	18.7	81.3	<0.1	>99.9
0.8	99.6	0.4	3.7	96.3	<0.1	>99.9
0.7	96.3	3.7	0.4	99.6	<0.1	>99.9
0.6	81.3	18.7	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

TNT in Soil

Analytical Confidence Factor	@ 0.5 □ Action Level		@ 1.0 □ Action Level		@ 2.0 □ Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	18.4	81.6	<0.1	>99.9
0.8	99.6	0.4	3.6	96.4	<0.1	>99.9
0.7	96.4	3.6	0.3	99.7	<0.1	>99.9
0.6	81.6	18.4	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 □ Action Level and false negative rates 0.1% or less at 2.0 □ Action Level. For example, applying a 0.8 *Analytical Confidence Factor (D)* (see worksheet on p. 4.5) for a TNT soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 99.6% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

CYCLODIENES RaPID ASSAY CHARACTERISTICS

Table 1 - Reactivity Factors for Cyclodienes RaPID Assay

Compound	Reactivity Factor
Dieldrin	1.00
Aldrin	1.25
Isodrin	1.04
Endrin	0.81
Heptachlor	0.62
Heptachlor-endo-epoxide	0.56
Chlordane	0.37
Endosulfan	0.22
Isobenzan	0.22
Toxaphene	0.09
Lindane	0.01

Table 2 - Analytical Confidence Factor Data

Cyclodienes in Water

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	>99.9	<0.1	20.2	79.8	<0.1	>99.9
0.8	99.4	0.6	4.8	95.2	<0.1	>99.9
0.7	95.2	4.8	0.6	99.4	<0.1	>99.9
0.6	79.8	20.3	<0.1	>99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Cyclodienes in Soil

Analytical Confidence Factor	@ 0.5 X Action Level		@ 1.0 X Action Level		@ 2.0 X Action Level	
	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
0.9	99.9	0.1	22.4	77.6	<0.1	>99.9
0.8	98.8	1.2	6.4	93.6	<0.1	>99.9
0.7	93.6	6.4	1.2	98.8	<0.1	>99.9
0.6	77.6	22.4	0.1	99.9	<0.1	>99.9
0.5	50.0	50.0	<0.1	>99.9	<0.1	>99.9

Note - The shaded data achieves false positive rates of about 10% or less at 0.5 X Action Level and false negative rates 0.1% or less at 2.0 X Action Level. For example, applying a 0.8 Analytical Confidence Factor (D) (see worksheet on p. 4.5) for a Cyclodienes soil analysis with an Action Level at 10 ppm would suggest that samples at 5 ppm (0.5 X Action Level) would give a negative immunoassay results 98.8% of the time. Samples at 20 ppm (2.0 X Action Level) would give a positive results greater than 99.9% of the time.

WORKSHEET

Cutoff Concentration _____ (A)

Standard 2 Concentration _____ (B)
(from Table 1)

Target Dilution Factor (A/B) _____ (C)

Kit Sample Dilution _____ (D)
(from Table 1)

Dilution Factor (C/D) _____ (E)

Volume of *Working Sample* _____
(from Column III of Dilution
Chart, p. 5.4)

Volume of Sample Diluent _____
(from Column II of Dilution Chart)

Table 1 - SDI Kit Information

Analyte	Standard 2 Concentration (ppb)	Sample Matrix	Kit Sample Dilution
PCP	2 ppb	Water Soil	1 1000
PCB	1 ppb	Water Soil	2 2000
TNT	1.0 ppb	Water Soil	1 1000
Cyclodienes	7.5 ppb	Water Soil	1.33 100

Example -

To optimize the PCB RaPID Assay system for a 40 ppm in soil cutoff (Step numbers refer to the Protocol step numbers given earlier in the General Procedure section):

- 1) *Cutoff Concentration* _____ 40,000 ppb (A)
- 2) *Standard 2 Concentration* _____ 1 ppb (B)
- 3) *Target Dilution Factor (A/B)* _____ 40,000 (C)
- 4) *Kit Sample Dilution* _____ 2,000 (D)
- 5) *Dilution Factor (C/D)* _____ 20 (E)

From Dilution Chart (p. 5.4):

- Volume of *Working Sample* _____ 100 µL
- Volume of Sample Diluent _____ 1.9 mL
- 7) If the result on diluted sample from RaPID Assay is _____ 2.3 ppb (F)
 - 8) Then the concentration of sample in original matrix is $(F \times C)$ _____ 92,000 ppb (G)
 - 9) Compare (G) to *Cutoff Concentration* (A); greater is _____ Positive for
positive, less negative _____ Action Level

SHORT OPERATING PROCEDURE FOR THE RPA-I

ALLOW THE RPA-I TO WARM UP FOR 30 MINUTES PRIOR TO USE. Avoid analyzing samples with air bubbles, foam, scratches, or foreign matter. The RPA-I performs a self test first. If all parameters are satisfactory, the "Select Command" prompt will appear. If there are abnormalities, an "Error" message will appear.

The RPA-I reports all results on a thermal paper printout. The unit is turned off by switching the power switch in the rear of the unit to the off position.

INSTRUMENT DISPLAY

SELECT COMMAND

RUN PROTOCOL: Aldicarb, Atrazine,
Alachlor, etc.

SPL. REPLICATES:
(1-5)

BLANK TUBE
INSERT TUBE



EVALUATING TUBE
REMOVE TUBE (Beep)

CAL. #1 REP. #1
INSERT TUBE

EVALUATING TUBE
REMOVE TUBE (Beep)

OPERATOR RESPONSE

Press RUN

Scroll using the YES [] or NO [] until the desired protocol appears. Press ENTER.

Press 1 (Press 2 if analyzing samples in duplicate, etc.). Press ENTER.

Insert tube with 1 mL of washing solution/buffer

Remove tube

Insert first standard replicate (0 ppb calibrator/tube #1).

Remove Tube

Follow the prompts on the instrument display.

Note: Tube order is important here. The RPA-I expects to see the standards/calibrators in ascending order in duplicate, starting with 0 ppb.

After all the standards (calibrators) have been evaluated, the instrument will display:

PRINTING DATA

Data will print.

LISTING XFORM
DATA

PRINTING CURVE

Curve will print only if programmed to print (See Section 3 Special Functions - Instrument Functions: Print Curve).

CTRL. #1 REP. #1
INSERT TUBE

Insert Control Tube.

EVALUATING TUBE
REMOVE TUBE (Beep)

Remove Tube.

EDIT CALIBRATORS
YES/NO

Press NO if it is not necessary to edit the calibrators, press YES to edit (See Section 3 Run).

SPL. #1 REP. #1
INSERT TUBE

Insert first Sample Tube.

EVALUATING TUBE
REMOVE TUBE (Beep)

Remove Tube.

Follow the prompts on the instrument display. After all the samples have been evaluated, press STOP.