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Storm Water Monitoring & Research Program

Caltrans Monitoring Protocols Guidance Manual: Toxicity

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California Department of Transportation

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SECTION 1
INTRODUCTION

This manual presents guidance for Caltrans management, staff, and contractors to use in the planning and implementation of toxicity studies for stormwater monitoring projects. The manual is designed and organized to provide step-by-step descriptions of the processes used to plan and implement successful toxicity studies specific to runoff from transportation-related facilities.

The main objective of this manual is to provide consistency in toxicity study methods among Caltrans toxicity investigations, as well as consistency in Caltrans toxicity study protocols over time. Such consistency is essential to provide for data comparability, and for ease of data entry in the Caltrans stormwater database. In addition to consistency of toxicity study methods, it is essential that toxicity data be collected so as to ensure that the data are accurate and precise. This manual therefore features detailed information on quality assurance and quality control procedures as they relate to toxicity testing.

Toxicity testing is considered a supplemental monitoring approach that can be used to support water quality monitoring, used to assess potential impacts of stormwater constituents to aquatic systems. Toxicity testing can be performed to determine if stormwater runoff is toxic prior to entering receiving waters, and additional testing can be performed to determine the fate (e.g. magnitude, areal extent, persistence) of this toxicity in the receiving water. Caltrans conducted a critical review of urban and roadway stormwater toxicity data (Caltrans, 1999a) which was used to formulate a recommended program for use of acute and chronic toxicity testing for Caltrans stormwater research and monitoring programs (Caltrans, 1999b). Existing stormwater toxicity data indicates a lack of consistency in the application of toxicity bioassays and use of a wide variety of differing toxicity test protocols. Use of United States Environmental Protection Agency (U.S. EPA) acute and chronic toxicity testing methods (U.S. EPA, 1993a, 1994) coupled with phased Toxicity Identification Evaluations (TIEs) (U.S. EPA, 1991, 1992, 1993b, 1993c) provides a standardized approach, including Quality Assurance/Quality Control (QA/QC), which allows for interpretation and application of toxicity testing results within and between studies. These standardized techniques have been applied for approximately the last ten years.

This guidance manual is specifically prepared to address the toxicity studies recommended by Caltrans and any future studies that may be designed to evaluate stormwater runoff from transportation facilities. Methods for addressing receiving water toxicity are not discussed in detail in this document. Protocols for receiving water toxicity studies may be added to this manual in the future as needed. Many of the components involved in planning and implementing toxicity studies are similar to those presented in the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). Therefore, this manual is organized in the same manner, with minor

variations, as the Stormwater Monitoring Protocols document, including two principal parts:

PART I: PREPARING THE STUDY PLAN

Part I of the manual is comprised of sections 2 through 6, covering topics relevant to planning toxicity studies, including: developing purpose and objectives, site selection, testing type selection, selection of sample collection methods and equipment, and documentation.

PART II: IMPLEMENTING THE STUDY PLAN

Part II of the manual is comprised of sections 7 through 10, covering topics relevant to implementing a toxicity study plan, including: sample collection, quality assurance/quality control, laboratory sample preparation and analytical methods, QA/QC data evaluation, and data reporting.

Training and preparation and logistics are additional components of implementing any stormwater study. Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) provides details concerning training and preparation and logistics for stormwater studies. These sections of the water quality document should be consulted for guidance when conducting Caltrans toxicity studies.

SECTION 2
DEVELOP PURPOSE AND OBJECTIVES

This section of the manual outlines the systematic decision-making process necessary to plan a Caltrans toxicity study that will achieve project-specific goals and objectives, including the development of appropriate DQOs. The process includes the following seven steps that summarize the systematic planning process (U.S. EPA, 2000):

- **Determine Project Goals and Objectives**
- **Identify Resources and Constraints**
- **Identify Data Characteristics and Tools**
- **Determine Key Study Parameters**
- **Specify Methods for Obtaining Data**
- **Develop Performance/Acceptance Criteria**
- **Optimize the Design for Obtaining the Data**

This process is illustrated in Figure 2-1 and each step of the process is discussed in the following subsections.

Throughout this planning process is it essential to bear in mind that runoff from transportation facilities is generally episodic in nature, and in most cases is storm-driven. The study design must therefore account for the sporadic and transitory nature of the runoff events.

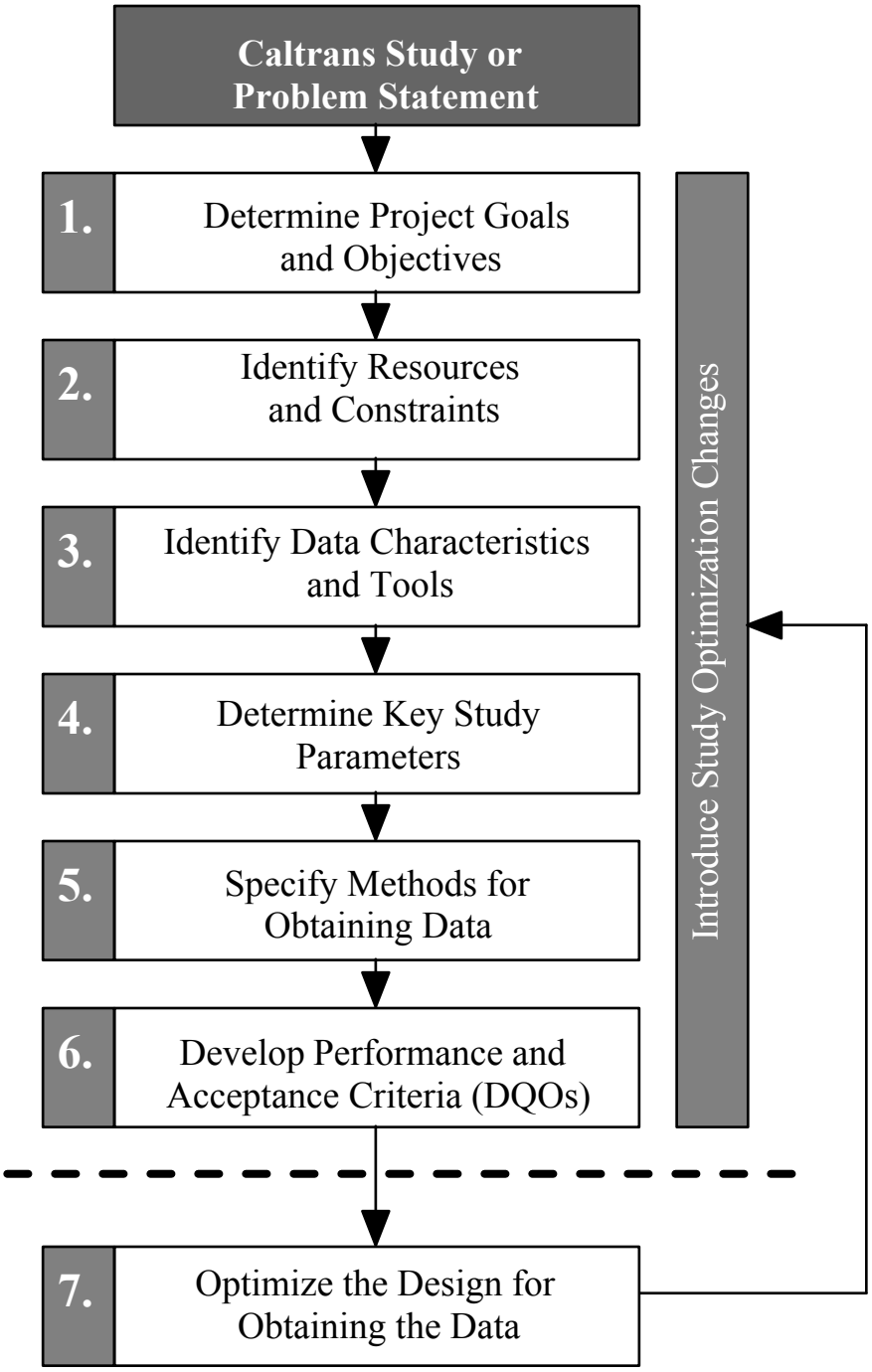

Figure 2-1. Systematic Planning Process Flow Chart

DETERMINE PROJECT GOALS AND OBJECTIVES

The overall goal(s) of the study are used to determine the specific questions that the study will be designed to answer; from these questions, specific study objectives are developed, as discussed below.

Determine Project Goal

The initial step in the development of a toxicity study plan is to determine the goal of the project based on the task order or problem statement presented by Caltrans. The overall goal of the study will in most cases have been defined previously in the Caltrans Stormwater Management Plan (SWMP) and other planning documents (e.g. Recommendations on the Use of Acute and Chronic Toxicity Testing for Caltrans Stormwater Research and Monitoring Programs, Caltrans 2000b).

Once the goal of the study is well defined, the project purpose can be stated so as to direct the project to provide data to fulfill the study goal. The goal and purpose of the study are strongly linked, and should be explicitly stated in the study plan. The purpose of the study is, generally speaking, to provide data in fulfillment of the study goals. Once the program goals and purpose are established, a set of specific study questions and objectives should be developed.

Determine Specific Study Questions/Objectives

The project goal(s) should be used to develop specific questions that the toxicity study will be designed to answer. These study questions are also in many cases previously defined in the Caltrans SWMP and other Caltrans planning documents. Specific monitoring project objectives are then developed to provide answers to the specific study questions.

Any applicable regulatory or legal program requirements must be considered in formulating the key question(s) to be used in defining project objectives. The study questions and project objectives must be specific enough to provide the basis for a detailed study design.

A primary objective of Caltrans toxicity studies will be to confirm and document the extent and cause(s) of toxicity of Caltrans stormwater discharges. This can be accomplished using a variety of approaches, depending on the nature of the specific study objectives. Typical questions that may be applied to Caltrans toxicity study projects include:

1. **Is runoff toxic to test organisms at any point during the runoff hydrograph?** In practical terms, this question asks whether the runoff is toxic at its maximum effect during a runoff event. As stormwater runoff commonly is thought to contain the highest concentrations of constituents in the initial or "first flush"

portion of the event, testing of first flush samples is often the focal point of this question.

2. **Is there a change in runoff toxicity over the course of the hydrograph?** This question pertains to the degree to which runoff toxicity varies during an event. Related studies may focus on fixed time intervals, or on discrete segments of the runoff hydrograph (i.e., the first flush, during the ascending limb, at peak flow, during the descending limb).

3. **Throughout the course of a runoff event as a whole, is runoff toxic?** Because runoff from transportation facilities is typically episodic and of relatively short duration, it may be necessary to evaluate the "average" level of toxicity associated with exposure to runoff over the course of the entire event storm or runoff event.

4. **What is the cause of the observed toxicity?** Given a demonstrated toxic effect, this question seeks to identify the cause(s). Toxicity identification evaluations are typically performed to address this question.

Sample collection approaches appropriate to address questions 1-3 are discussed in detail in ***Section 7***, question number 4 is discussed in ***Section 4***.

Other potential objectives of Caltrans toxicity studies may be related BMP effectiveness, land use types, and seasonal and/or other temporal trends. Specifically the following questions could be incorporated into toxicity studies:

5. **Are there differences in the toxicity of runoff from different locations or land uses?** Because runoff from different sources may contain differing levels of potentially toxic substances, studies may be needed to quantify related differences in toxic effect.

6. **Does BMP implementation reduce toxicity?** Toxicity testing can be an important tool for evaluating BMP performance, as toxicity study data can be used to determine if a given BMP actually removes the causes of toxicity in runoff. Knowledge of stormwater toxicity and of specific toxic agents can also aid in effective selection of stormwater BMPs, either through implementation of source controls for toxic constituents, or by the design and implementation of structural controls targeted at removal of specific toxics (Caltrans, 1999b).

7. **Are there longer-term seasonal (within a year) or temporal (between years) trends in toxicity?** Runoff quality is known to vary significantly from event to event (depending on the hydrological characteristics of the event), within and between seasons (depending upon factors related to the seasonal build-up and wash-off of pollutants, such as the antecedent dry period and cumulative rainfall to date), and from year to year (especially when comparing wet and dry years). It may be necessary to quantify the relative level and nature of toxic responses to runoff from these various temporal conditions.

Once study questions and objectives are known, specific project information needs are identified. The information needs must address any pertinent legal (court-ordered) or regulatory requirements (as specified in the NPDES permit or mandated through other state or federal regulations), and focus the study planning effort on providing answers to the key question(s) addressed by the project.

IDENTIFY RESOURCES AND CONSTRAINTS

The specific resources available and constraints pertaining to implementing the toxicity study should then be identified. Planned studies are often supported by data resources from previous studies, as well as other existing resources such as personnel, equipment, and services. Studies are typically constrained by time, available budget, physical limitations of the study area, and practical weather constraints (e.g., number of storms in a year). Toxicity studies can be further complicated by availability of test species. These resources and constraints must be well established during the planning process. At the start, the systematic planning process assumes that the fiscal constraints on a project are not primary. Practically speaking, budget constraints are known from the beginning and generally affect the scope and detail of the study. The project may also be constrained by applicable regulatory or legal requirements.

IDENTIFY DATA CHARACTERISTICS AND TOOLS

The study design should specify the expected characteristics of the data and the planned data analysis tools, so as to:

- optimize collection of data within the financial and logistical/practical limitations of the proposed study, and
- ensure that useful results will be provided to fulfill study objectives.

For example, for a TIE study the quantity and quality of the data should be sufficient to develop a clear picture of the characteristics of the toxicity (i.e. constituents, concentrations, and duration). This may equate to monitoring fewer sites (i.e., selecting representative sites) to develop detailed data, instead of monitoring a large number of sites to merely confirm that stormwater runoff is toxic.

Essential data characteristics include the type of toxicity data to be collected (e.g., screening level, acute and/or chronic), the variables affecting the data (e.g., antecedent conditions, rainfall intensity, site type and location), and the expected variability of the data (derived from previous studies when available).

Prior to the initiation of a toxicity study, a strategy should be developed for analysis of the data, directed to answering the specific study questions. The selected data analysis technique(s) may influence the type and quantities of data required to satisfy study objectives. The analysis methods applied to data collected for BMP evaluations or

characterization studies typically involve straight-forward statistical operations; however, Caltrans data may be applied to multiple applications in the future (e.g., modeling applications, comparisons to other studies, and assessments of toxicity in receiving waters), and data should be collected and presented in a manner that will allow the most versatile use of the data (see ***Section 10***).

DETERMINE KEY STUDY PARAMETERS

Key parameters of the toxicity study are determined using the information gathered in the previous steps of the systematic planning process. Key study parameters include site selection (see ***Section 3***), number of monitored storm events and their temporal distribution, characteristics of target storm events, types of samples (composite, grab, etc.) and toxicity test method (see ***Section 4***). The better these characteristics are understood, the more efficiently the monitoring data can be collected.

The monitoring locations can be selected to optimize the physical monitoring of the environmental sample stream(s); this is discussed in more detail in ***Section 3***. If possible, sites selected for toxicity studies should be monitoring stations in place for other programs which will generate supporting chemical data.

The planned numbers of sites and monitoring events are often constrained by fiscal factors; this involves not only the costs of sample collection, but also the costs of sample analysis. For this reason, the number and types of toxicity tests should be considered in the early stages of project planning (see ***Section 4***), so the costs of the appropriate sample collection and analysis can be factored into the expected cost per monitoring event.

The types of storms to be monitored and optimal temporal distribution of monitoring events also should be considered during project planning. For example, if the project objectives include characterizing seasonal variation in stormwater toxicity, the monitoring events must be adequately distributed throughout the period(s) of interest. Besides seasonal distribution, a number of variables can be considered when selecting types of storms to monitor. These variables could include storm size (rainfall amount), duration, and antecedent conditions (such as number of days since the previous rainfall). Storm selection criteria should be developed to guide project managers in the appropriate selection of monitoring events during the monitoring season.

In addition to considering the types of storms to monitor, the timing of sample collection (as it relates to the event hydrograph) should also be considered. Possible approaches may include collection of a single grab sample during the initial or “first flush” portion of the event, collection of single grabs during discrete portions of the hydrograph (ascending, peak, and/or descending portions), collection of multiple grab samples at points throughout the event, or collection of an event-length composite sample.

SPECIFY METHODS FOR OBTAINING DATA

Once the key study parameters are known, appropriate data collection methods are specified (see ***Sections 5, 7 and 9***). This includes the types of samples collected and sample collection techniques (flow-based composite, automated sampling, grab, etc.), the equipment used to collect the samples, the toxicity testing methods, QA/QC procedures, and the specific procedures to be followed in accordance with the physical layout of the study site (e.g., which discharge flows best characterize the highway runoff and the practical considerations for collecting samples at that site). The sample collection methods must consider the method specifications for sample handling, containers, sample type, holding time, and preservation (see ***Sections 5*** and ***9***).

DEVELOP PERFORMANCE/ACCEPTANCE CRITERIA

The central goal of any toxicity study is to provide scientifically defensible data in fulfillment of program objectives. This goal is best achieved through scientific design of the study and sound technical planning. Statistical methods may be used in the design of the study to determine the optimum number of sampling sites and sampling events (monitoring frequency) that will be needed to fulfill specific project objectives. Performance acceptance criteria, also known as DQOs, should be specified for the project, to help ensure that the data produced by the study will be useful in addressing study objectives, and to establish meaningful confidence levels for the monitoring data.

DQOs, as defined for this guidance document, specify the quality of data required to support the specified objectives of toxicity studies. DQOs generally are used to determine the level of error considered to be acceptable in the data produced by the study; in large measure they are used to specify acceptable ranges of field sampling and laboratory performance. The DQOs should be specified in the project Data Quality Evaluation Plan (DQEP) (see ***Section 9***) and referenced in the Sampling and Analysis Plan (SAP).

A detailed discussion of DQOs for Caltrans stormwater monitoring projects is presented in the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). In general, the same systematic approach for determining DQOs for stormwater monitoring projects applies to establishing DQOs for toxicity studies. For the purposes of this Guidance Manual, DQOs refer to the set of specific performance and acceptance criteria applied to field and laboratory activities associated with toxicity studies. The DQOs developed for each project establish measures of acceptable performance and data quality for the collected data, and this guidance manual describes procedures by which toxicity data should be generated and evaluated.

USEPA methods for toxicity testing outline test acceptability criteria as well as specifications for test conditions. These parameters are discussed in detail in ***Sections 8 and 9***. Any failure to meet test acceptability criteria or any deviation from test

specifications must be noted and data must be evaluated in light of these deviations (*see* ***Section 8***).

Analytical precision and accuracy are often determined by historical laboratory performance; in some cases interlaboratory studies conducted by USEPA or others are used to set these limits. Each project should include a thorough QA/QC data review and evaluation to assess project success in meeting accuracy and precision acceptance criteria (see ***Section 8***). Specifically, the following QA/QC parameters should be reviewed:

- Holding times
- Test acceptability criteria
- Contamination check results (field, bottle, and equipment blanks)
- Precision analysis (laboratory duplicates, reference toxicants)

Representativeness criteria for testing of event-length composite samples are discussed in detail in ***Section 7*** and are based on storm size, percent storm capture, and total number of composite sample aliquots.

OPTIMIZE THE DESIGN FOR OBTAINING THE DATA

Ideally, the systematic planning process can be iterative for each project, as needed. It is possible to modify study parameters, with approval from the Caltrans task order manager, to optimize data collection to meet study objectives, based on newly collected data and field observations. This can sometimes be done during natural break points of the study (e.g., at the end of the wet season), when data are analyzed and achievement of study objectives is assessed. In some cases, the collected information may indicate modification of the study goals, the collection methods, the toxicity test type or species, or the types and frequency of runoff events sampled.

The systematic planning process, as applied to the Caltrans Statewide Stormwater Monitoring Program, is a synthesis of currently evolving EPA guidance and the practical considerations of overall goals (Caltrans, 2000a). The seven-step process described in this section is the starting point for structuring specific Caltrans toxicity studies. The remaining sections of this manual carry out and build on these steps, from site selection to QA/QC data evaluation. The seven step process is iterative, with the final step providing for feedback to any of the previous six steps.

SECTION 3
SITE SELECTION

Selection of sites for Caltrans toxicity studies should start with evaluation of monitoring stations that exist for other programs (e.g. existing Caltrans stormwater monitoring locations). If possible these existing sites should be selected for toxicity studies to save time and resources and to provide supporting chemical data. Some modifications and use of different sample collection methods may be required at existing runoff monitoring sites to accommodate the larger sample volumes needed for toxicity testing (see ***Section 7***). Site selection will also depend on the program objectives and any regulatory or legal requirements. However, once these criteria have been used to establish the number and type(s) of monitoring sites, consideration of the following items will help ensure selection of the most appropriate monitoring locations for toxicity studies:

- **Representativeness**
- **Best Management Practices (BMP) Effectiveness**
- **Site Visit**

This section focuses on use of pre-existing Caltrans runoff monitoring sites for toxicity study sample collection. Stormwater monitoring program site selection is discussed in detail in Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a), including information on the above items as well as personal safety, site access, equipment security, flow measurement capability, electrical power and telephone, and non-Caltrans sources. Site selection for receiving water studies is not included in this manual.

REPRESENTATIVENESS

Types of runoff sampling sites specified for Caltrans monitoring may include highway sites (freeways, expressways and/or conventional highways), maintenance yards, park-and-ride lots, or construction sites. It is important to select specific monitoring sites that are representative of typical Caltrans operations for these site types. The Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) provides guidance on site characteristics to consider when selecting representative monitoring sites.

For toxicity studies it may be useful to select sites that discharge directly into identifiable receiving water bodies. This would support future toxicity testing that may be conducted to investigate the potential receiving water impacts of Caltrans discharges.

BMP EFFECTIVENESS

Studies of BMP effectiveness involve different objectives than those for general stormwater discharge monitoring. The goals of BMP monitoring programs are somewhat different as well. The Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) provides detailed information concerning the selection of sites for BMP performance monitoring.

Site selection for BMP evaluation will depend upon the type of BMP (e.g. structural or non-structural). Structural BMPs typically have well-defined boundaries and may be relatively easy to monitor. When evaluating structural BMPs, monitoring locations should be located immediately upstream and downstream of the structure. This will allow for comparison between the toxicity of stormwater influent of the BMP and stormwater effluent of the BMP.

Non-structural BMPs (e.g. street sweeping, catch basin cleaning, illicit discharge elimination) may be more difficult to monitor because they can be influenced by many factors that cannot be "controlled", and may not have clearly defined inlet and outlet flows. In such cases, it may be necessary to determine the toxicity of runoff at the same sites before and after BMP implementation.

It is assumed that any toxicity testing used to measure BMP effectiveness would be conducted on sampling stations from existing Caltrans BMP pilot studies.

SITE VISIT

Each potential monitoring site should be visited to confirm the expected site characteristics and verify whether the site is suitable for the needs of the toxicity study. When possible, a visit should be conducted during or after a storm, when the discharge flow conditions can be observed. A wet-weather visit can provide valuable information regarding logistical constraints that may not be readily apparent during dry weather. However, a dry weather visit should also be conducted to observe any non-stormwater flows. If any significant dry weather flows are present this should be noted and considered during final site selection.

Because some types of toxicity tests require large sample volumes, especially if TIE follow-up is planned, the sample station equipment should be evaluated to ensure that adequate sample collection is possible. See ***Section 9*** for sample volume requirements.

SECTION 4
TESTING METHOD SELECTION

To select the specific type of toxicity tests to be conducted for a given toxicity study, the following items should be considered:

- **Project Objectives and Resources**
- **Regulatory and Legal Requirements**
- **Pollutant Sources in the Catchment Area**
- **Existing Monitoring Data**
- **Beneficial Uses/Impairments of the Receiving Water**
- **Constituents to Be Used for Data Interpretation**
- **Typical Toxicity Methods**

Each of these considerations is discussed below, followed by a list of the suite of possible toxicity tests Caltrans may use for toxicity studies.

A summary schematic of the process followed in determining the types of tests to be conducted is shown in the flow chart in Figure 4-1. The procedure calls for examining the purpose and goals of the project, legal requirements, existing permits, and any expected or likely toxicants. Consideration of sampling and analysis costs (i.e., available project resources) is included as a final step in making this determination, as most projects are fiscally constrained.

PROJECT OBJECTIVES AND RESOURCES

The types and number of toxicity tests selected for a given study will ultimately depend upon the objectives and available resources (e.g. personnel, funds, time). As discussed in the Recommendations on the Use of Acute and Chronic Toxicity Testing for Caltrans Stormwater Research and Monitoring Programs (Caltrans, 1999b), primary purposes of Caltrans toxicity studies may be to:

- Confirm and document the extent of toxicity of Caltrans stormwater discharges;
- Identify constituents causing toxicity in Caltrans stormwater discharges;
- Determine the effectiveness of BMPs in the removal of toxicity from Caltrans stormwater discharges;

- Interpret toxicity data with respect to potential receiving water impacts; and
- Determine toxicity/bioaccumulation effects of stormwater sediments deposited into receiving waters.

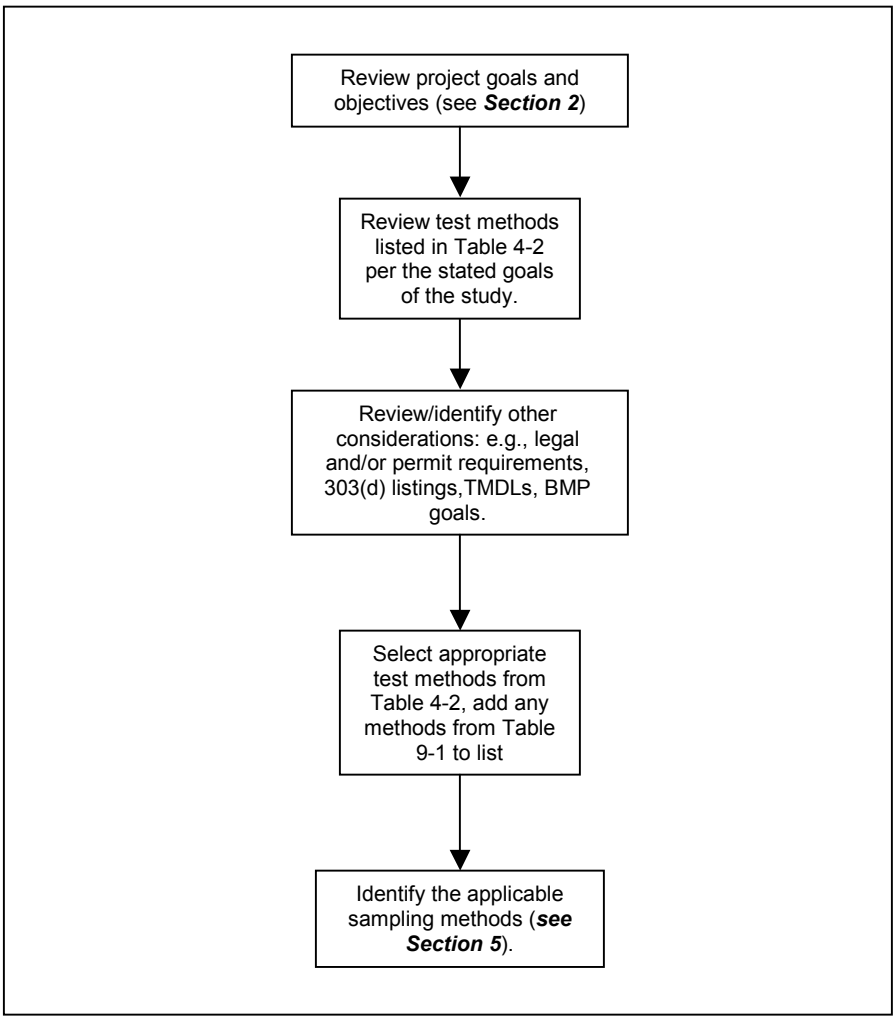

Figure 4-1. Flow chart of toxicity test method selection process.

REGULATORY AND LEGAL REQUIREMENTS

Currently, specific toxicity requirements are not outlined in the Caltrans Statewide Stormwater NPDES permit. The permit does state that discharges shall not cause or contribute to violations of water quality objectives nor shall they cause certain conditions to occur which create a condition of nuisance or water quality impairment in receiving waters. As a permitted discharger, Caltrans may be obligated to fulfill related requirements under state or federal regulations, specifically the State Water Code or the Clean Water Act. Caltrans also may be subject to relevant court ordered legal requirements.

POLLUTANT SOURCES IN THE CATCHMENT AREA

Identifying potential constituent sources in the catchment area can aid in identification of potential toxicants and the selection of toxicity test methods to be utilized. Caltrans projects may involve monitoring of runoff from highways, maintenance yards, park-and-ride lots, and construction sites. The potential constituent sources associated with each of these facilities may differ. A detailed description of the types of constituents expected from highway runoff, maintenance yards, rest areas, park-n-ride lots, and construction sites is provided in Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).

If particular constituents are expected from certain catchment areas, this may help in the selection of the species tested and may guide TIE efforts. According to toxicity studies conducted by others, for land-use categories including urban residential, commercial, or light industrial most of the observed toxicity has been attributed to organophosphate insecticides (e.g. diazinon, chlorpyrifos). Evidence has included analytical chemistry and TIE confirmation (See Table 4-1). A limited number of studies have focused on runoff predominately or exclusively from roadways or highways (See Table 4-1).

As shown in Table 4-1, a review of the literature revealed that both urban and highway stormwater samples caused toxicity due to concentrations and combinations of metals, non-polar organic compounds, and/or suspended solids. Salt compounds used for deicing roads, and organic compounds used for deicing aircraft were both found to be toxic in standardized bioassay tests. However, in several cases, the toxicant was not identified. Additionally, toxicity associated with highway runoff samples was related to a number of factors, including the traffic load (average daily traffic (ADT)) and the length of time between storms that flushed materials from roads (Caltrans, 1999b).

Table 4-1. Stormwater Runoff Toxicity: Urban/Suburban/Mixed and Transportation Related Runoff Studies

Author (Year)	Location	Land Use/ Sample Source	Species[1]	Bioassay Type	Results/Suspect Toxicants
URBAN/SUBURBAN/MIXED					
BASMAA (1996)	CA-SF Bay Area	Industrial	***Ceriodaphnia*** *Pimephales* *Selenastrum*	Chronic Chronic Chronic	*Ceriodaphnia* toxicity was attributed to diazinon, non-polar organics
ToxScan (1998)	CA-Stockton	Urban (sumps)	***Ceriodaphnia*** ***Selenastrum***	Acute Chronic	Diazinon, malathion (*C.dubia*) non-polar organics, Zn (algae)
ToxScan (1996a)	CA-Stockton	Urban (sumps)	***Ceriodaphnia***	Acute	Diazinon, chlorpyrifos, malathion, weak evidence of volatile/surfactant and metals
ToxScan (1995a,b)	CA-Sacramento	Reservoir and river samples	*Ceriodaphnia*	Chronic	No toxicity observed
ToxScan (1993a,c)	CA-Sacramento	Urban creek	***Ceriodaphnia***	Acute	Non-polar organics, metals
ToxScan (1993b)	CA-Sacramento	Urban creek and river samples	*Ceriodaphnia* *Pimephales* *Selenastrum*	Chronic Chronic Chronic	See Toxscan 1993 a,c (TIEs performed for *C.dubia* only. Slight reduction in growth was observed for *P. promelas* and *Selenastrum* showed no effects)
ToxScan (1992a)	CA-Sacramento	Urban creek and river samples	***Ceriodaphnia*** *Pimephales* *Selenastrum*	Chronic Chronic Chronic	TIEs were not conducted. All river samples were toxic to *C.dubia*, river samples were not toxic. *P. promelas* growth was effected in both river and creek samples. River and creek samples effected Selenastrum growth, creek samples were more toxic.
ToxScan (1996b,c)	CA- Contra Costa	Urban stream	*Ceriodaphnia*	Chronic	TIEs not conducted. Both *C.dubia* survival and reproduction were effected.
ToxScan (1995c,d, e)	CA- Contra Costa	Urban stream	*Ceriodaphnia*	Chronic	TIEs not conducted. Both *C.dubia* survival and reproduction were effected.
ToxScan (1994a,b,c)	CA- Contra Costa	Urban stream	*Ceriodaphnia*	Chronic	TIEs not conducted. Both *C.dubia* survival and reproduction were effected.
Schiff & Stevenson (1996)	CA-San Diego	Urban creek	*Ceriodaphnia*	Chronic	TIEs not conducted. *C.dubia* reproduction was effected.

Table 4-1. cont.

Author (Year)	Location	Land Use/ Sample Source	Species[1]	Bioassay Type	Results/Suspect Toxicants
SCCWRP (1996)	CA- SoCal	Urban creek compared to undeveloped area	*Haliotis* ***Strongylocent -rotus***	Chronic Chronic	Urban creek samples were consistently more toxic. TIE results presented in Jirik et al. 1998 (below)
Jirik et al. (1998)	CA- SoCal	Urban creek compared to undeveloped area	***Strongylocent -rotus***	Chronic	Zn, Cu
Lopes & Fossum (1995)	AZ	Mixed use	***Pimephales*** *Ceriodaphnia*	Acute Acute	Surfactants/organics, Cu & Zn (*P. promelas*) Samples not toxic to *C.dubia*
Medeiros et al. (1984)	MA	Urban stream	*Pimephales*	Chronic	TIEs not conducted.
TRANSPORTATION					
Bogdanic (1997)	CA- SF Bay Area	Construction sites and freeway	*Pimephales*	Acute	No toxicity observed
BASMAA (1996)	CA- SF Bay Area	Two highway sources	***Ceriodaphnia*** *Pimephales* *Selenastrum*	Chronic Chronic Chronic	Non-polar organics, Metallo-organics
Fisher et al. (1995)	MD	Airport	*Pimephales* ***Daphnids***	Acute Acute	One episode of toxicity to daphnids; Ethylene glycol suspect toxicant
Lord (1987)	US	Highways with various Average Daily Traffic (ADT) from 12,000 to 120,000	*Pimephales* *Selenastrum* *Gammarus* *Hexagenia* *Daphnia*	Acute Chronic Acute Acute Acute	Metals (*Selenastrum*). Toxicants not determined for other species. No toxic response to *P. promelas* or daphnids. Some effect to *Gammarus* and *Hexagenia* but not related to ADT.
Portele et al. (1982)	WA	Highways with various ADT BMP-grass swale	*Selenastrum* *Daphnia* *Oncorynchus*	Chronic Acute Acute	Toxicity to *Selenastrum* observed at one site. Daphnia toxicity at one site; filtration of sample did not change toxicity. Toxicity to *Oncorynchus* observed for unfiltered samples but not filtered samples or samples that passed through grass swale.
Little et al. (1982)	WA	Highway	***Selenastrum***	Chronic	Pb, Cu, Zn
Winters and Gidley (1980)	CA	Highway	**Indigenous Algae**	Chronic	Zn, Pb (metals) Worst inhibition of growth at high traffic volume (185,000 ADT). Lower ADT and freeway samples with short dry period were stimulatory.
Caltrans	Districts 6, 8 and 10		*Ceriodaphnia*	Acute	Not reported

Table 4-1. cont.

Author (Year)	Location	Land Use/ Sample Source	Species[1]	Bioassay Type	Results/Suspect Toxicants
Caltrans (UC Davis, 1999)	CA – Modesto	Agricultural, urban, highway	*Ceriodaphnia* *Pimephales* *Selenastrum* *Oncorynchus*	Acute and Chronic	Diazinon (*C. dubia*), None identified (*P. promelas*)
Caltrans	Statewide	Caltrans facilities including rest areas, maintenance yards, highway, and park-and-rides.	*Ceriodaphnia* *Pimephales* *Selenastrum*	Chronic Chronic Chronic	Toxicity was observed at all but 2 sites. Non-polar organics implicated in 9 *P.promelas* and 9 *C. dubia* TIEs, metals implicated in 4 *P.promelas* and 1 *C.dubia* TIEs. Metabolically activated pesticide implicated in 9 *C.dubia* TIEs. The pesticide was suggested for highway sites, park-and-ride, and maintenance yards. The cause of *C.dubia* toxicity was not identified for 5 of the sites.
Caltrans	CA – Navaro		*Ceriodaphnia* *Pimephales* *Selenastrum*	Chronic Chronic Chronic	Not reported

1 If applicable, the most sensitive species and/or species tested in TIEs are highlighted in bold.

EXISTING TOXICITY DATA

Table 4-1 provides a summary of the results of stormwater runoff toxicity studies conducted by Caltrans and others, including information on the species tested, test duration (i.e. acute or chronic) and significant findings of TIEs.

The existing data from these previous studies forms the essential basis for the list of Caltrans toxicity test types specified in Table 4-2. Note that the list of toxicity test types applies to Caltrans runoff studies only, and therefore assumes a freshwater matrix. In some cases it may be prudent or necessary to add additional test methods, for example to address legal requirements or specific receiving water concerns.

BENEFICIAL USES/IMPAIRMENTS OF THE RECEIVING WATER

Receiving water toxicity studies are not discussed in detail in this document, but information may be incorporated into this document once Caltrans begins to evaluate toxicity in receiving waters. In turn, this document focuses on freshwater species only; which will be applicable for use for freshwater receiving water studies.

Toxicity data on stormwater discharges, and on dilutions with the receiving water, can give a more direct measure of potential beneficial use impacts than water chemistry data alone. Toxicity test organisms integrate the individual toxicants biologically to provide effects-based response information, as opposed to inferred responses from chemical constituent data. Toxicity data are therefore useful to demonstrate whether beneficial uses are being protected. For such purposes, data will be required so that results can be interpreted for both freshwater and marine receiving waters of importance to California. Marine and estuarine species must be considered if toxicity studies are conducted to evaluate marine and estuarine receiving waters. The effects of dilution by receiving waters, the toxicity in the receiving water from runoff from non-roadway sources, and the concept of exposure duration also need to be considered (Caltrans, 1999b).

Table 4-2. List of Freshwater Toxicity Test Types[1]

Bioassay Type	Species	Duration	Method	Endpoints Measured	Description/Application
Zooplankton	▪ *Ceriodaphnia dubia*	Acute (24, 48 or 96 hrs)	EPA/600/4-90/027	Survival	*Ceriodaphnia* has been shown to be sensitive to stormwater in many studies throughout California (SCCWRP, 1999). This species represents a taxonomic group (crustacean) that is an important member of freshwater aquatic communities.
Fish	▪ *Pimephales promelas* (Fathead Minnow) ▪ *Oncorhynchus mykiss* (Rainbow Trout)[2]	Acute	EPA/600/4-90/027	Survival	Direct link to beneficial uses and these species represent a taxonomic group (teleost) that is an important member of freshwater aquatic communities.
Zooplankton	▪ *Ceriodaphnia dubia*	Chronic (7 days)	EPA/600/4-91/002	Survival, reproductive success, behavioral changes	*Ceriodaphnia* has been shown to be sensitive to stormwater toxicity in many studies throughout California (SCCWRP, 1999). This species represents a taxonomic group (crustacean) that is an important member of freshwater aquatic communities.
Fish	▪ *Pimephales promelas* (Fathead minnow)	Chronic (7 days)	EPA/600/4-91/002	Survival, growth (weight), behavioral changes	Direct link to beneficial uses and these species represent a taxonomic group (teleost) that is an important member of freshwater aquatic communities.
Algal	▪ *Selenastrum capricornutum*[3]	Chronic (96 hrs)	EPA/600/4-91/002	Growth	Growth is measured as an indicator of eutrophication potential. Also, certain toxicants could suppress growth and testing could be structured to get insights on the relative influences of nutrients and toxicants in diverse water samples (Horner, 1997).

1 Listed tests include the freshwater tests most commonly used in past transportation-related runoff studies.
2 Rainbow trout were used by Caltrans to determine developmental toxicity to an indigenous species.
3 Species name has been changed to *Raphidocelis subcapitata*.

CONSTITUENTS TO BE MEASURED TO SUPPORT TOXICITY TEST DATA

Field Measurements

At the time of toxicity sample collection, field crews should measure temperature, pH, dissolved oxygen (DO), and electrical conductivity. Methods and equipment for measurement of these parameters are discussed in ***Sections 5 and 7.*** Measurement of these parameters in the field can be used to assess changes in these parameters from the time of sample collection to the time of test initiation. This information may be valuable in determining potential changes in toxicity (see discussion below).

Laboratory Measurements

USEPA toxicity testing methods specify limits for temperature, dissolved oxygen (DO), and pH values during testing. Variations in these parameters in the test solutions can cause changes in the toxicity of the sample and are therefore monitored closely to ensure measurements are within the specified limits. The measurements required by U.S. EPA methods are as follows:

Adverse effects of low DO concentrations, high concentrations of suspended and/or dissolved solids, and extremes of pH, alkalinity, or hardness, may mask the presence of toxic substances.

Acute tests:

- At a minimum, pH and conductivity are measured in the sample and the control.
- It is also recommended that total alkalinity and total hardness be measured in the sample and the control.
- DO should be measured in the sample prior to use and should be near saturation. See ***Section 9*** for information on sample aeration.

Chronic tests:

- DO is measured at the beginning and end of each 24-hour exposure period in at least one test chamber at each test concentration and in the control. See ***Section 9*** for information on sample aeration.
- Temperature and pH are measured at the end of each 24-hour exposure period in at least one test chamber at each test concentration and in the control.
- Temperature should also be recorded continuously or observed and recorded daily for at least two locations in the environmental control system or the samples. Temperature should be measured in a sufficient number of test vessels at least at the end of the test to determine the temperature variation in the environmental chamber.

- pH is measured in the sample each day before test solutions are made.
- It is also recommended that conductivity, total alkalinity, and total hardness be measured in each sample and the control.

U.S. EPA toxicity testing protocols recommend measurement of total ammonia and residual chlorine; however, this may not be necessary for stormwater samples unless these are suspect toxicants (i.e. measurement of these constituents is included in EPA guidance which was developed primarily to characterize municipal and industrial effluents, which can contain significant concentrations of these constituents).

Specialized chemical analyses may be conducted on selected toxicological samples to assist in the characterization and confirmation of specific compounds that may be responsible for stormwater toxicity. For example, if the TIE process identifies pesticides as the primary toxicants, the toxic sample can be analyzed for pesticide concentrations. However, it is important to be aware of sample holding times and other requirements (e.g. preservation) for chemical analyses.

TYPICAL TOXICITY METHODS

It is common to test stormwater samples using both acute and chronic test procedures. Both acute and chronic toxicity tests have been used in transportation related stormwater runoff studies (see Table 4-1), most commonly using the standard three freshwater species *Ceriodaphia dubia* (zooplankton), *Pimephales promelas* (fish), and *Selenastrum capricornutum*[1] (algae, chronic only). Based on a review of the existing data, the initial toxicity study approach suggested by Caltrans (1999b) recommended use of three-species chronic protocols to establish the most sensitive species for subsequent monitoring. Additionally, it was recommended that screening-level bioassays (using undiluted runoff samples) be used initially, so that the existence of toxicity can be confirmed and characterized in the most cost-effective manner.

Identification of the causative agents in toxic samples can be performed utilizing TIE methods outlined by U.S. EPA (1991, 1993a, 1993b). Phased TIEs include a set of procedures for characterizing the physical/chemical characteristics of the toxicants in a sample, as well as the variability associated with the type and concentration of compounds that cause toxicity in the sample.

A brief summary of acute testing versus chronic testing, screening level versus dilution tests, selection of appropriate species, and TIEs is presented below.

Details of each toxicity test method are provided in ***Section 9.***

[1] This species name has been changed to *Raphidocelis subcapitata*.

Acute vs. Chronic

There are advantages and disadvantages to both acute and chronic toxicity testing. The main advantage of acute testing is cost (typically less than half the cost of chronic). Acute tests provide a more immediate result than chronic tests, but also provide data on only one endpoint (survival). Chronic toxicity tests are considered more sensitive because they focus on other endpoints (e.g. reproductive success, growth) in addition to survival.

Acute vs. Chronic Toxicity

Acute toxicity describes the adverse effects resulting from a single or short-term exposure to a substance. The exposure period for most acute toxicity tests is less than 96 hours, depending on the test species. Lethality is the endpoint measured, but sublethal effects such as behavior or physical defects are also recorded.

Chronic toxicity describes the adverse effects from repeated exposures to a substance over a longer period of time. The exposure period for most chronic toxicity tests is 4-7 days. The effect can be measured in lethality, impaired growth, reduced reproduction, etc.

It may appear that acute toxicity tests would be more appropriate for stormwater samples because runoff events are typically relatively short-term events. However, chronic tests may be appropriate in instances where events occur frequently (e.g. multiple storms per month). This is true because some of the effects of stormwater discharges are associated with organic and toxic pollutant accumulations over a long time and are not associated with individual runoff events (Field and Pitt, 1990 as cited in Johnson et al. 1998).

Depending upon the objectives and available resources of a given toxicity study, one possible approach might include conducting acute tests initially to determine if acute toxicity exists; this would avoid wasting the costs of setting up a chronic test. If acute toxicity is not observed, subsequent testing could be conducted under chronic exposure conditions.

Screening Level vs. Dilution Tests

Stormwater samples collected for toxicity testing may be tested either at the full (100%) concentration only (screening test) or in a dilution series. Screening level tests may be considered (either acute or chronic) as an initial approach to determining runoff toxicity. This approach is the most cost-effective to confirm the existence of toxicity in runoff from Caltrans conveyance systems. Dilution series tests could then be conducted on samples from sites that demonstrated consistent and significant toxicity during screening. Testing a dilution series on samples from these sites would allow for quantification of the toxic response.

Selection of Test Species

The following criteria may be used to assist in selection of appropriate test species (SCCWRP, 1999):

- Sensitivity to stormwater toxicants
- Comparability with prior or planned data
- Representativeness of local fauna or key animal groups
- Suitability for TIE methods

It is also important to consult with the laboratory conducting the testing to determine if the selected species is easily cultured in the laboratory and/or is available throughout the year from commercial sources. Additionally, indigenous species may only be used if culturing and testing methods have been developed for the selected species. However, EPA allows the use of indigenous species only where state regulations require their use or prohibit importation of the species provided by EPA guidance (EPA, 1993). Use of indigenous species other than those recommended by EPA guidance requires side-by-side toxicity tests with reference toxicants and/or runoff samples to ensure that the species selected are at least as sensitive as the recommended species.

TIEs

Identification of the constituents present in stormwater samples causing toxicity is an important component of toxicity studies. Identification of toxicants in runoff from Caltrans conveyances may provide information on the origin of the toxicants, and suggest whether they are products of transportation-related activities or whether they are deposited by rainfall or dustfall. Toxicant identification may also help in the design and effectiveness evaluation of BMPs for toxicant control.

U.S. EPA has developed a series of procedures (Phase I, II, and III TIEs) for characterizing, identifying, and confirming the identity of suspect toxicants in toxic samples. The phased TIE approach develop by U.S. EPA seeks to identify toxicants in a sample via a series of manipulations and associated bioassays which target a systematically narrowing list of suspect toxicants. Phase I TIE tests are used to determine the nature of the components causing toxicity and to identify the possible chemical category of the toxicant(s) (e.g. metals, nonpolar organics, volatiles). Once the likely chemical category is identified Phase II manipulations are used to identify the specific toxicant(s). Additional monitoring data (e.g. physical and chemical, pollutant source) may provide information that can be used to direct Phase II testing. Phase III is used to confirm that the suspect toxicant(s) identified in Phase II is/are indeed the true toxicant(s).

TIE methods were initially developed to identify toxicants in acutely toxic samples. However, Phase I TIE methods for chronically toxic freshwater samples have been

published by U.S. EPA (U.S. EPA, 1992). Phase II and III chronic methodologies are incorporated into respective Phase II and III TIE manuals (U.S. EPA, 1993b, 1993c).

TIE methods should only be applied after toxicity has been observed in prior toxicity tests and should be applied to the same species showing the toxic response. To conduct TIEs in the most efficient and cost-effective manner possible, the approach should be outlined and discussed with the laboratory prior to initiation of a toxicity study. At a minimum the following items should be considered:

- Toxic signal – samples which demonstrate a high level of toxicity are preferable, since reduction of that toxicity after manipulation is easily measured and quantified. The level of toxicity required to trigger a TIE should be discussed with the laboratory (e.g. TIEs will only be performed on tests where a 50% reduction from the control is observed).

- Loss of toxicity – TIEs require that toxicity be present and that it not be rapidly degraded. For example, some samples demonstrate high initial toxicity which rapidly decays during storage thus preventing successful TIE characterization. This may especially be a concern for longer-term chronic effects, that is, if seven day tests are required for initial toxicity assessment and for toxicity evaluations and TIE manipulations, the toxicant must be more stable and persist longer in storage.

- Constituents of potential concern – if certain constituents are expected to be present at a given site this may help guide TIE efforts. For example, if high concentrations of metals are expected TIE manipulations specific to identifying the presence of metals can be performed in initial TIE efforts. Alternately, if certain constituents are not expected in stormwater samples it may be appropriate to develop an abbreviated subset of U.S. EPA procedures. For example, Phase I TIE procedures were designed for the characterization of municipal and industrial effluents and therefore include manipulations that target constituents expected in these types of effluents (e.g. ammonia, chlorine). These manipulations may not be appropriate for stormwater samples.

- Existing Data – Results of past TIEs conducted with stormwater samples may also help guide initial TIE efforts. Past investigations reviewed by Caltrans (Caltrans, 1999b) show that the toxicant groups that should be targeted in Phase I TIEs include volatile or oxidizable compounds, particulate-bound toxins, cationic metals, non-polar organics, and organophosphates.

Additional information on specific TIE manipulations is provided in Appendix A.

SECTION 5
SAMPLE COLLECTION METHODS AND EQUIPMENT

This section discusses the various methods and equipment that may be used for collection of samples for toxicity testing. This section is organized under the following subsections:

- **Sample Collection Methods**
- **Sample Collection Equipment**

Water quality in stormwater runoff may vary both laterally and vertically throughout the cross section of flow, and with time. For instance, floatable materials (oil, grease, light particles and debris, scum) may be present in significant amounts near the water surface, while heavier sediments are often concentrated near the bottom of the conveyance. Also, concentrations of some constituents may be higher in the first hour or two of runoff; this is often referred to as a "first flush" effect. During the course of a storm, rainfall intensity also may increase, raising runoff flow rates to the point where sediments are mobilized and scour occurs, resulting in temporarily higher concentrations of sediment-bound constituents.

Toxicity studies should be designed to ensure that collected samples are representative of runoff quality during the period of concern, based on the project objectives. For example, collection of samples to determine if runoff is toxic throughout an entire runoff event is best done by collection of flow-proportioned composite samples throughout the runoff hydrograph. See ***Appendix C*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for a discussion of flow-proportional compositing. Multiple grab samples may also be collected and combined into one composite sample for the same purpose. Studies may be designed to evaluate the toxicity of a specific part of the storm, this means collection of a discrete grab sample at a designated time during the storm. Finally, samples may be collected to determine if there is a change in toxicity over the hydrograph. This means collection and testing of multiple grab samples at key points throughout the hydrograph (at a minimum at first flush, during ascending limb, at peak flow, and during descending limb). Grab and composite sample collection methods and equipment are discussed below.

Flow measurement and precipitation measurement provide useful information to support stromwater runoff sample collection (e.g. flow measurement is necessary for accurate sample compositing and precipitation data can be used to understand site hydrology). Other important components of successful collection of stormwater runoff samples include system integration, system command/control, and remote communication. Each of these components are discussed in detail in Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). This section discusses the sample methods and equipment specific to collection of stormwater runoff samples for toxicity testing.

SAMPLE COLLECTION METHODS

Stormwater samples can be collected to represent a point in time (i.e., "grab" sample) or a period of time (i.e., "composite" sample). A grab sample is essentially a one-time collection of a sample volume adequate to perform the intended water quality analysis or toxicity tests. A composite sample is comprised of some number of individual sample aliquots mixed together. For stormwater monitoring, this usually refers to samples collected sequentially over time during a stormwater runoff event.

Stormwater samples can be collected using either manual or automated means. Project designers should select the combination of sample types and techniques most suitable to the toxicity study objectives and available resources.

Both automated composite sampling and manual grab sampling are appropriate for toxicity sample collection, depending on the study objectives. The advantages and disadvantages of each sample collection method in terms of toxicity testing are provided in Table 5-1.

Table 5-1. Advantages and Disadvantages of Grab and Composite Samples

Sample Type	Advantages	Disadvantages
Grab	▪ Easy to collect; requires a minimum of equipment. ▪ Provide a measure of instantaneous toxicity. Toxicity spikes are not masked by dilution.	▪ The chances of detecting a spike in toxicity would depend on the timing and frequency of sampling; the probability of missing toxicity spikes is high.
Composite	▪ The sample is collected over a longer period of time than grab samples and may contain toxicity spikes. ▪ Can provide a measure of toxicity throughout the event.	▪ Automated sampling equipment is sophisticated and expensive. ▪ Toxicity spikes may not be detected because they may be masked by dilution with less toxic runoff.

Composite Sampling

Automated sampling generally is the most cost-effective method of composite sample collection, and is particularly appropriate for large-scale programs (e.g., where a large number of sampling sites are monitored, or numerous sampling events are conducted over multiple years).

Automated monitoring systems are comprised of electromechanical devices that can be operated automatically (i.e., an on-site operator may not be needed). Automated sample collection is recommended for the following reasons:

- On-site time requirements by personnel are reduced during sample collection; therefore, the threat to worker safety by hazardous conditions (e.g., traffic, inadequate oxygen, toxic or explosive gases) is decreased.

- Automatic samplers can be programmed so that sampling operations are initiated when pre-determined conditions are met, such as a specific flow rate, flow depth, or rainfall amount. This reduces the possibility of missing the early stages of runoff.
- Automated equipment can be programmed in conjunction with a flow meter to collect flow-weighted composite samples, using a constant volume proportional flow method (see ***Appendix C*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)). This permits collection of representative samples throughout the storm event.

Composite samples also may be collected using manual methods. This approach may be warranted when the toxicity study is designed to evaluate toxicity at certain points of the storm or where the installation of automated sampling equipment is economically or logistically infeasible, or is not capable of providing the required sample volume. Although manual composite sample collection is more labor-intensive during a storm event, it will reduce required station setup and maintenance prior to each storm event. See ***Section 7*** for a description of manual compositing techniques.

Grab Sample Collection

A grab sample is an individual sample collected at one specific site at one point in time. Analysis of a grab sample provides a "snapshot" of stormwater quality. Grab samples are most often collected using manual methods and equipment (e.g. bailers, grab poles, etc.). Grab samples for toxicity testing can be collected directly into the bottle that will be used in the laboratory or an intermediate container may be used to fill the sample bottle (e.g. plastic cubitainers are most easily filled by collecting the samples in a glass amber bottle and pouring from this into the cubitainer). Grab sample containers must be filled to the top, leaving no head space to minimize the loss of toxicity due to volatilization of toxic constituents. See ***Section 9*** for additional information on sample container types.

SAMPLE COLLECTION EQUIPMENT

For all collection efforts, sampling devices must be made of chemical-resistant materials that will not affect the quality of the stormwater sample. In general, suitable sampling devices should be constructed of one of three materials: Teflon, glass, or polyethylene. These materials are known to be the most inert in terms of adsorption or desorption of organic and inorganic compounds. It is important to evaluate each component of the sampling device (e.g., bottle lids, connections, tubing) for possible sources of sample contamination. For example, latex and neoprene rubber tubings should be avoided because latex is organic and neoprene rubber may contain trace metals. Butyl rubber seals may also contribute organics to the sample.

Equipment that is constructed of stainless steel, such as dippers or sample intake strainers, may be Teflon-coated to make them suitable for use in collection of stormwater samples for toxicity testing.

Selection of Sample Bottle Type

Selection of the most appropriate sample container will depend upon study objectives (i.e. inclusion of TIEs), sampling logistics, and what is know about possible toxic constituents at each site. For toxicity testing both polyethylene "cubitainers" and borosilicate glass bottles have been used for sample collection. Selection of the most appropriate sample container will depend upon the following:

- Study objectives (i.e. inclusion of TIEs),
- Sampling logistics,
- What is known about possible toxic constituents at each site.

U.S. EPA recommends glass containers rather than plastic when TIEs are planned. U.S. EPA has reported that for some samples, toxicity was greater and was retained longer in samples collected and stored in glass bottles. This effect was attributed to adsorption of certain types of toxicants (e.g. surfactants, organophosphate insecticides) to polyethylene containers (U.S. EPA, 1991)).

Logistically, use of glass bottles creates some difficulty as transportation of large sample volumes in glass containers requires extra care (i.e. field crews must ensure that samples are packed effectively to prevent breakage during shipment to the laboratory).

If information is available regarding the targeted constituents at a given site, this should guide the selection of sample containers. For example, if metals are suspect toxicants it is more appropriate to collect samples in polyethylene containers. At sites where organics are of concern, glass is preferred over polyethylene. The use of polyethylene containers for toxicity samples has been shown to reduce toxicity when cationic polymers are present (i.e. the charged surface of plastic containers reduced toxicity). Polycarbonate has been identified as an alterative to polyethylene because it is less reactive to organics. Selection of appropriate sample containers for a given toxicity study should be evaluated and discussed with the laboratory prior to initiation of the study.

Automated Sampling Equipment

The key elements of an automated monitoring system that are required to collect flow-proportioned composite samples include:

<u>Sample Collection</u>:

- An automatic sampler,
- Sample intake tubing and strainer, and
- Sample bottle(s).

Flow Measurement:

- A flow measurement device,
- Flow sensor and cable, and
- Optional rain gauge.

Data Recording/Retrieval:

- A data storage device,
- System software,
- Optional remote communications capabilities, and
- Optional secondary/back-up power source.

These components are discussed in detail in the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).

Key components required for automated sample collection include an automatic sampler, tubing with strainer, and sample bottle(s); these components are further described below.

Automatic Sampler

Automatic samplers are comprised of a peristaltic pump, pump control electronics, a sample distribution system, a power supply, and a housing that contains the composite bottle(s). A peristaltic pump creates suction by compressing a flexible tube with a rotating roller, drawing a sample that is then pushed out of the pump. The pump operates best when placed close to the sample source; this reduces the suction head or lift (experience has shown that the reliability of peristaltic pumps drawing a consistent sample volume is greatly reduced as the static suction head increases). According to manufacturer's specifications, vertical lift must be no greater than 26 feet. When sampling with an automated sampler, static head height refers to the vertical distance from the surface of the flow stream to the automatic sampler pump inlet.

Automatic samplers may be configured for single or multiple bottle composite collection. Samplers configured to fill multiple sample bottles have a sample distribution mechanism. Tubing from the discharge port of the intake pump is connected to a rotating distributor arm that dispenses the samples into several sample bottles.

If flow-weighted sampling is planned, the automatic sampler must be capable of accepting a signal from a flow meter that is used to trigger collection of a sample aliquot. Cables can be purchased that connect each type of flow meter to an automated sampler. Additional details concerning automatic samplers are provided in the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)

Sampler Intake Strainer, Intake Tubing and Flexible Pump Tubing

The intake strainer is attached to the intake tubing and mounted to the bottom of a pipe or channel. Intake strainers prevent rocks and debris from clogging or damaging the intake tubing or pump. Sizes vary, but smaller intake strainers are generally better for use under low flow conditions. Sample intake strainers are typically made of stainless steel, or a combination of stainless steel and Teflon. For collection of samples for toxicity testing, all intake strainer parts must be Teflon, or coated with Teflon or Teflon-like material. Specific intake and pump tubing requirements and guidance on intake strainer placement is presented in Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).

Sample Bottle(s)

Automatic samplers may be configured to have a variety of sample bottles, from one to a dozen or more sample bottles. If a sampler holds a single bottle, all of the sample aliquots are pumped into this bottle, resulting in one large composite sample. Use of a single composite bottle does not allow for isolation of specific samples or groups of samples from specific periods of the runoff hydrograph, and provides less visual indication of sampler malfunction (if this should occur). A multiple bottle configuration, however, provides these capabilities.

It is important to keep extra bottles (for either the single or multiple bottle configuration) available in case bottles are contaminated or damaged, or in the event that bottles need to be changed to accommodate a larger-than-expected storm. If a storm delivers more precipitation than expected, sample bottles will fill prior to the end of the storm, prompting sample bottle replacement. Additional bottles may also be needed when large volumes of sample are required by the toxicity tests type selected.

In-situ Analytical Measurement Equipment

Certain conventional water quality parameters, including temperature, pH, electrical conductivity, and dissolved oxygen, should be measured in the field at the time of sample collection to provide basic indicators of the quality of the sample and to provide information to assess changes between time of collection and test initiation (see ***Section 4***). Electronic equipment is available to measure these parameters. Electronic sensors and data loggers may allow for near-continuous measurements of indicator parameters at reasonable cost. This equipment also makes it feasible to monitor every storm that occurs over a period of time, including short-duration events that would otherwise be impractical to monitor. In addition, data reduction and evaluation are simplified because the water quality results are simply downloaded from the data logger onto a laptop computer or a personal computer with a modem. However, it is important to realize the limitations associated with the use of sensors and data loggers:

- Sensors are only available to measure a limited number of parameters.

- The ion-specific sensors now available may not provide low enough detection limits to meet monitoring project DQOs.
- It may be difficult to assure the quality (i.e., precision & accuracy) and reliability of the results.
- Laboratory analysis is required in conjunction with the results to allow correlations to be made between the indicator readings and concentrations of specific constituents of concern.
- It may not be possible to reliably correlate a sensor-measured "indicator" parameter with a given constituent of concern.
- Sensors are subject to fouling or clogging from debris, algae, oil & grease, etc.
- Some sensors (e.g., turbidity) may require substantial flow for accurate readings.
- Sensors require routine maintenance to ensure proper function.
- Some sensors (e.g., pH, DO, conductivity) cannot be allowed to dry out; this is problematic in many drainage conveyances.

Manual Sampling Equipment

At some sampling locations it may be necessary to use manual sample collection equipment. Manual equipment can include hand-operated items such as bailers, grab poles, pitchers, or other items designed to lower an appropriate sample container into the runoff flow. This equipment is relatively inexpensive and easy to maintain. Additional information concerning manual sample collection and equipment in provided in ***Section 7***.

SECTION 6
SAMPLING AND ANALYSIS PLAN (SAP)

Proper documentation is required for all Caltrans toxicity studies. A stormwater SAP and Health and Safety Plan (HSP) should be prepared prior to initiation of any sampling activities.

This section provides general guidance for preparation of practical, useable SAPs for stormwater monitoring programs for toxicity testing. See ***Appendix D*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for detailed information regarding individual components to be included in the HSP.

The following are sections that should be included in a SAP:

- **Project Overview/Description**
- **Sampling Site(s)**
- **Toxicity Tests to be Conducted**
- **Data Quality Objectives (DQOs)**
- **Monitoring Preparation and Logistics**
- **Sample Collection, Preservation, and Delivery**
- **Quality Assurance/Quality Control**
- **Laboratory Sample Preparation and Analytical Methods**
- **Data Management and Reporting Procedures**

Appendix:

- **Health and Safety Plan (HSP)**

Inclusion of these sections will ensure development of a useable and practical SAP. The final product should be a single bound document that can be easily referenced during monitoring activities. This can be conveniently done by including the HSP as an appendix to the SAP. Printing the SAP/HSP on waterproof paper will reduce the potential for water damage to the document during field use.

All Caltrans stormwater monitoring documentation must be assigned a document identification number prior to document completion. The document identification number, assigned by the Stormwater Management Program document distribution system manager, is used to track the document and develop document distribution lists. Two weeks prior to expected document deliverable date, the author must contact the Caltrans stormwater management program document distribution system manager to receive a document identification number and mailing labels.

The SAP should be prepared by someone with a good understanding of field sampling procedures, laboratory testing methods, and data validation procedures. The toxicity laboratory should provide input to ensure that the SAP (especially the QA/QC portion) is realistic, and consistent with the laboratory's operating procedures. Field personnel should also provide input regarding logistical details, and ensure that the plan is practical and easy to use.

The SAP should include a thorough description of all activities required to implement the toxicity study. The plan should be organized to provide an overview of the project goals and organization, followed by a description of all monitoring activities in the chronological sequence in which they will typically occur. The plan should specify the quality assurance/quality control protocols that will be followed by field and laboratory personnel, and how the field and laboratory results will be managed and reported.

The contents of a typical stormwater SAP are shown in Figure 6-1.

1.0 PROJECT OVERVIEW/DESCRIPTION
- 1.1 Description of why the project is being conducted
- 1.2 Description of who is conducting the project
- 1.3 General scope of monitoring activities
- 1.4 Project organization/roles and responsibilities

2.0 SAMPLING SITE(S)
- 2.1 Site location (map) including Global Positioning System (GPS) coordinates
- 2.2 Written driving directions
- 2.3 Site access instructions (gates, locks, keys, combinations)
- 2.4 Notification procedures

3.0 TOXICITY TESTING TYPES
- 3.1 Identification of Methods (include SOPs for each)

4.0 DATA QUALITY OBJECTIVES (DQOs)
- 4.1 Analytical reporting limits
- 4.2 Analytical precision, accuracy, and completeness
- 4.3 Data Quality Evaluation Plan

5.0 FIELD EQUPMENT MAINTENANCE
- 5.1 Equipment calibration
- 5.2 Equipment maintenance
- 5.3 Equipment cleaning (bottles/lids/tubing)

6.0 MONITORING PREPARATION AND LOGISTICS
- 6.1 Weather tracking
- 6.2 Storm selection criteria
- 6.3 Storm action levels
- 6.4 Communications/notification procedures
- 6.5 Sample bottle order
- 6.6 Sample bottle labeling
- 6.7 Field equipment preparation

7.0 SAMPLE COLLECTION, PRESERVATION, AND DELIVERY
- 7.1 Sample collection methods
- 7.2 Field measurement methods
- 7.3 Field equipment list
- 7.4 Sample containers, preservation, and handling
- 7.5 QA/QC sample collection methods
- 7.6 Sample labeling (site names/codes, etc.)
- 7.7 Composite sample splitting
- 7.8 Forms and procedures for documenting sample collection and field measurements
- 7.9 Laboratory communication procedures
- 7.10 Sample shipping/delivery, chain-of-custody

8.0 QUALITY ASSURANCE/QUALITY CONTROL
Field procedures for QA/QC sample collection

9.0 LABORATORY SAMPLE PREPARATION AND ANALYTICAL METHODS
- 9.1 Laboratory sample preparation procedures
- 9.2 Toxicity Testing Methods (including testing methods, sample handling, and holding times)

10.0 DATA MANAGEMENT AND REPORTING PROCEDURES
- 10.1 Data validation
- 10.2 Electronic data transfer
- 10.3 Filing of electronic and hard copy data
- 10.4 Reports

APPENDIX
A Health and Safety Plan

Figure 6-1. A Typical Stormwater Sampling and Analysis Plan Outline

The following sub-sections further describe the contents of a typical stormwater SAP that is applicable for toxicity studies.

OVERVIEW: PROJECT DESCRIPTION, ORGANIZATION, AND RESPONSIBILIES

This section of the SAP should briefly describe:

- Description of why the toxicity study is being conducted,
- Description of who is conducting the study,
- General scope of toxicity monitoring activities, and
- Roles and responsibilities of the key field and laboratory personnel who will be involved in the study (typical roles include project manager, sampling coordinator, sampling team leaders, field crew, project QA/QC officer, and laboratory QA/QC manager).

SAMPLING SITES

This section should describe the sampling sites selected for the toxicity study and provide rationale for the inclusion of a site. Site selection for toxicity studies should focus on existing Caltrans stormwater monitoring sites which were selected based upon representativeness of land uses in the area, achievable flow measurement, site access, personnel safety, equipment security, and availability of electrical power and telephone. See ***Section 3*** for monitoring site selection guidance.

The following detailed information should be included for each monitoring site:

- Site location map,
- GPS coordinates,
- Written driving directions,
- Site access instructions (locked gate keys or combinations), and
- Notification procedures (if notification is required prior to site access).

TOXICITY TEST METHODS

This section should specify the types of toxicity tests to be conducted to meet program objectives. See ***Section 4*** for toxicity test type selection guidance. The SAP should include a list of test types, in tabular form, that includes at a minimum the following columns:

- Species to be tested,
- Test duration (specify acute or chronic),
- Exposure concentrations,
- Sample collection method (i.e. grab or composite),
- Bottle type,
- Volume required,
- Preservation, and
- Laboratory performing testing.

U.S. EPA methods (1993a, 1994) provide summaries of test conditions for each toxicity test method, these protocols should be included in this section (see ***Section 9***).

DATA QUALITY OBJECTIVES (DQOs)

This section should specify the project goals and objectives, key project parameters, and the DQOs that were deemed appropriate for the project. See ***Section 2*** for guidance on the development of DQOs. DQOs should be outlined in a Data Quality Evaluation Plan (DQEP). The DQEP should also describe the process by which data produced by the study are evaluated to provide a reference point from which a program-consistent QA/QC evaluation can be performed (see ***Section 8***).

FIELD EQUIPMENT MAINTENANCE

This section should include specific information concerning the maintenance of all sampling equipment. At a minimum, all equipment should be maintained according to manufacturer specifications. The following is a list of items that typically require regular maintenance:

- Automatic sampler – calibrate, check battery and check/replace desiccant,
- Intake tubing/strainer - inspect & clear or replace,
- Flow measuring device - calibrate, check battery and check/replace desiccant,
- Rain gauge - calibrate and check for debris, and
- Portable field instruments (e.g. pH meter) – calibrate.

Bottle and equipment cleaning should also be covered in this section of the SAP (see ***Appendix E*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for guidance on sample bottle and equipment cleaning procedures). In addition, if equipment is located in a confined space, this section should include a discussion on the acceptable confined space entry procedures. See ***Section 5*** and Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for guidance on the selection of sampling methods and equipment and detailed equipment installation and maintenance guidance.

MONITORING PREPARATION AND LOGISTICS

This section should describe the necessary pre-storm preparations including:

- Weather tracking
  -Procedures
  -Responsibilities
  -Obtaining quantity of precipitation forecast (QPF)
- Storm selection criteria
  -Stage of wet season
  -Antecedent conditions
  -Storm duration
  -Recommended minimum storm size
  -Logistical constraints

- Storm action levels
  - *-Non-monitoring*
  - *-Standby*
  - *-Pre-alert*
  - *-Alert*
  - *-Go*
  - *-Post-storm*

- Communication/notification Procedures
  - *-Telephone tree*

- Sample bottle order

- Sample bottle labeling

- Field equipment preparation
  - *-Inspect*
  - *-Calibrate*
  - *-Charge batteries*
  - *-Check/replace desiccant*

See Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for monitoring preparation and logistics guidance.

SAMPLE COLLECTION, PRESERVATION, AND DELIVERY

This section should provide detailed guidance on standard operating procedures for field personnel (i.e., a concise, useable guide for sampling activities). At a minimum, this section should include:

- Description of clean sample handling techniques,
- Sample collection methods to be used at each location,
- Field measurement equipment (e.g., pH meter) and methods to be used at each location,
- Field equipment that will be required for monitoring at each station (includes a checklist so field people can ensure they have everything they will need before proceeding to the sampling location),
- Sample labeling procedures (site name/code, sample date and time, etc.),
- Sample compositing and splitting procedures (including testing prioritization for instances of insufficient sample volume),

- Procedures for collecting and labeling field blanks and duplicate samples,
- Forms and procedures for documenting sample collection and field measurements,
- Sample preservation and handling procedures,
- Laboratory communication procedures, points of contact, and phone numbers,
- Procedure for sample shipment or delivery to laboratory, and
- Details on completing chain-of-custody forms.

See ***Section 7*** for detailed sample collection, preservation, and delivery guidance.

QUALITY ASSURANCE/QUALITY CONTROL

The QA/QC section should focus on QA/QC procedures necessary for field activities, such as field procedures and collection frequencies for the following:

- Laboratory duplicates
- Field blanks
- Equipment blanks

QA/QC sample collection frequencies are best presented in a QA/QC sample collection schedule table with a column for each event and a row for each site.

Laboratory QA/QC should be discussed in an appendix and should include the frequency and type of laboratory QA samples (e.g., laboratory duplicates, split samples, and reference toxicant tests), data reporting requirements (electronic and hard copy) and corrective actions. See ***Section 8*** for detailed QA/QC guidance.

LABORATORY SAMPLE PREPARATION AND TEST METHODS

This section should describe the activities to be conducted by the toxicity laboratory, including sample preparation procedures. A table containing the following information should be presented:

- Species to be tested,
- Test duration,
- Holding time,
- Test acceptability criteria, and
- Laboratory QA/QC

See ***Section 9*** for laboratory sample preparation and toxicity test methods guidance.

DATA MANAGEMENT AND REPORTING PROCEDURES

This section should describe how the data generated from the laboratory and field measurements are to be handled, evaluated, and reported. It should address:

- Data validation (QA/QC review in light of project DQOs, per procedures specified in the DQEP),
- Electronic data transfer,
- Filing of electronic and hard copy data,
- Data analysis (e.g., statistical evaluation, etc.), and
- Reports (e.g., quarterly, annual, end of project).

At the beginning of the project, the field and laboratory personnel should review the entire SAP to obtain an overview of their respective roles and responsibilities. Before each sampling event, the field personnel should review the sampling methods section in detail, and the laboratory personnel should review the laboratory sample preparation and methods section. See ***Section 8*** for QA/QC data evaluation guidance.

Efficient data storage, retrieval, and transfer methods should be established prior to initiation of any monitoring activities, including those to support toxicity studies. Suggestions are provided in the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) to guide Caltrans contractors and district personnel in the development of a reliable and useful data management system. Data gathered during toxicity studies will be incorporated into the Caltrans Statewide Stormwater Quality Database. Specific data reporting protocols for toxicity results are outline in ***Section 10***.

HEALTH AND SAFETY PLAN

Stormwater monitoring involves activities that have the potential to adversely affect the health and safety of field personnel. Stormwater monitoring field crews often work in wet, poor-visibility conditions. Sampling sites may be located along streets or stream channels, or in remote, poorly-lit areas, and may require access on a 24-hour basis. Workers may be exposed to traffic hazards, confined spaces, biological hazards, hazardous materials, fast-moving storm waters, and slippery conditions. Besides monitoring activities during storm events, monitoring equipment installation and maintenance activities may also take place under these conditions.

The information contained herein is for guidance only, and does not supersede or otherwise change any applicable state, local, or agency health and safety requirements or programs.

The general steps in HSP development for a given stormwater monitoring project are listed below:

- Assign a health and safety officer to oversee all health and safety activities.
- Write concise descriptions of all of the field activities that will be included in the program.
- Identify and evaluate the potential physical, chemical, and biological hazards associated with each activity.
- Specify the measures to be employed to reduce the health and safety risks associated with the identified hazards (i.e., precautions).
- Specify health and safety training and documentation requirements.
- Develop an emergency response/contingency plan.

Each of these steps is described in ***Appendix D*** of the Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). Figure 6-2 contains an example outline for a project-specific HSP.

1.0 Introduction

2.0 Project And Safety Personnel

3.0 Site Information

4.0 Work Activities Covered By Health And Safety Plan

5.0 Hazard Assessment
- 5.1 Chemical Hazards
- 5.2 Confined Spaces
- 5.3 Physical Hazards
- 5.4 Biological Hazards

6.0 General Health And Safety Requirements
- 6.1 Employee Clearance
- 6.2 Site Safety Meetings
- 6.3 Accident Reporting
- 6.4 Prohibited On-Site Activities
- 6.5 Communications

7.0 Site-Specific Health And Safety Requirements
- 7.1 Special Medical Tests
- 7.2 Special Training
- 7.3 Physical Hazards
- 7.4 Hazardous Materials Identification And Protection
- 7.5 Confined Space Entry
- 7.6 Traffic Control
- 7.7 Personal Protective Equipment
- 7.8 Site Illumination
- 7.9 Biological Hazard

8.0 Emergency Response Procedures
- 8.1 Hospital Information
- 8.2 Emergency Route to Hospital
- 8.3 First Aid & Related Equipment

Figure 6-2. A Typical Health and Safety Plan Outline

SECTION 7
SAMPLE COLLECTION

Equipment and bottles used in the collection of stormwater samples for toxicity testing must be handled with great care to minimize the possibility of contamination. The ease with which stormwater samples can be unintentionally contaminated cannot be overemphasized. Samples collected for toxicity testing must also be handled properly to avoid possible changes in the toxicity of the sample (e.g. loss of volatile organic compounds). The following procedures include sample collection and handling techniques that maximize the ability of sampling personnel to collect samples reliably and with minimal sample contamination or loss of toxicity.

The following are basic sample collection and handling elements required during collection of stormwater samples for toxicity testing:

- **Personnel Safety**
- **Sampling Equipment and Bottles**
- **Clean Sampling Techniques**
- **Grab Sample Collection**
- **Composite Sample Collection**
- **Flow Monitoring**
- **Composite Bottle Changing**
- **Field Measurements**
- **Sample Representativeness Evaluation**
- **Multi-bottle Compositing and Composite Sample Splitting**
- **Sample Preservation and Holding Time**
- **Sample Delivery/Chain of Custody**

These elements are described below to provide sample collection and handling guidance for field personnel engaged in stormwater monitoring.

PERSONNEL SAFETY

Before samples are collected, personnel must ensure the safety of such activities at each sampling location. As discussed in Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a), personnel safety should be considered when selecting monitoring sites. Adherence to the following recommendations will minimize risks to sampling personnel:

- At no time during storm conditions or when significant flows are present should sampling personnel enter a manhole or standpipe.
- Two-person field crews should be available for all field work to be conducted under adverse weather conditions, or whenever there are risks to personal safety.
- Use of automated samplers can eliminate many of the hazards associated with manual sample collection, as personnel may not be required to be at the site for composite sampling.
- Personnel must be trained regarding appropriate traffic control measures. If appropriate, a traffic control plan should be developed for each site and included in the sampling plan and analysis plan prior to conducting sampling events.
- Only personnel properly trained and equipped for confined space entry may conduct a "confined" space entry; this applies to both the entrant and the observers.
- When appropriate, an encroachment permit must be filed with the local Caltrans district.

SAMPLING EQUIPMENT AND BOTTLES

For toxicity studies, field personnel may be required to collect composite samples and/or grab samples. It is important to use the appropriate sample bottles and equipment for collection of toxicity samples (see ***Section 5***). Improper bottles and equipment can introduce contaminants and cause other errors which can invalidate the data. For example, chemicals may leach from the bottle into the sample, or waterborne constituents may adsorb to sampling equipment or to the sides of the bottle.

Sample Bottles

Polyethylene "cubitainers" or borosilicate glass bottles may be appropriate for toxicity samples, depending on the objectives of the study, sampling logistics, and what is known about possible toxic constituents at each site (see ***Section 5***). The selection of the appropriate sample bottle type should be discussed with the laboratory prior to initiation of the study. Refer to Table 9-1 in ***Section 9*** for additional information.

Equipment and Bottle Cleaning and Handling

Before samples are collected, all sampling equipment and bottles are cleaned in a laboratory using appropriate solvent rinses, deionized water and/or other methods (high temperatures). The laboratory is responsible for generating acceptable equipment blanks and sample bottle blanks to demonstrate that the sampling equipment and bottles are free from trace metals and organics contamination before they are delivered to field sampling personnel. An acceptable blank is one that is free from toxicity. ***Section 8*** provides additional information on collection of equipment blanks.

New plasticware used for sample collection or organism exposure vessels does not require thorough cleaning before use. New sample containers should be rinsed before use according to the protocol outlined in the bottle rinsing and labeling section below.

New glassware must be soaked overnight in 10% acid and rinsed well in deionized water and dilution water. All non-disposable sample containers, test vessels, tanks, and other equipment that have come in contact with effluent must be washed after use to remove contaminants as described below:

1. Soak 15 min in tap water and scrub with detergent, or clean in an automatic dishwasher.
2. Rinse twice with tap water.
3. Carefully rinse once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals and bases. To prepare a 10% solution of acid, add 10 mL of concentrated acid to 90 mL of deionized water.
4. Rinse twice with deionized water.
5. Rinse once with full-strength, pesticide-grade acetone to remove organic compounds (use a fume hood or canopy).
6. Rinse three times with deionized water.

After cleaning, sample bottles and laboratory-cleaned sampling equipment are handled only while wearing clean, powder-free nitrile gloves. All laboratory-cleaned sampling equipment is double bagged in clean zip-lock plastic bags for storage or shipment. Clean bottles are stored in a clean area with lids properly secured.

Bottle Labeling and Rinsing

Immediately prior to rinsing sample bottles, the bottle labels should be checked, and date and time added using a waterproof pen. Attempting to label grab sample bottles after sample rinsing and/or collection may be difficult because of wet labels.

Before stormwater samples are collected for toxicity testing, each sample bottle must be rinsed with site water according to the following procedure:

- Rinse every sample bottle **three** times with site water.
- For containers smaller than 1-liter, rinse each container by filling it with site water three times.
- For containers 1-liter and larger, fill one container with site water and use it to pour a little water into each of the other containers.
- If samples are poured from larger composite bottles, site water from the composite sample should be used for rinsing prior to filling the sample bottle.

- When rinsing each container be sure the cap is on the bottle so that the cap gets rinsed as well.

CLEAN SAMPLING TECHNIQUES

Caltrans stormwater monitoring projects employ "clean" sampling techniques to minimize potential sources of sample contamination, particularly from trace pollutants. Experience has shown that when clean sampling techniques are used, detected concentrations of constituents tend to be lower. Clean sampling techniques are especially important for toxicity analysis because tests are sensitive to the combined effect of all constituents in the sample. As a result, unmeasured and unidentified compounds that would not interfere with chemical analysis may interfere with toxicity tests. Clean sample collection techniques that should be followed during the collection of stormwater samples for toxicity testing are described below. More extensive clean sampling techniques may be required under certain conditions, such as monitoring to assess receiving water impacts.

Extreme care must be taken during all sampling operations to minimize exposure of the samples to human, atmospheric, and other potential sources of contamination. Care must be taken to avoid contamination whenever handling bottles, lids, sample tubing, and strainers. Whenever possible, grab samples should be collected by opening, filling and capping the sample bottle while submerged, to minimize exposure to airborne particulate matter. Bottles should be completely filled, leaving no head space. Additionally, whenever possible, samples should be collected upstream and upwind of sampling personnel to minimize introduction of contaminants.

To reduce potential contamination, sample collection personnel must adhere to the following rules while collecting stormwater samples:

- No smoking
- Always wear clean, powder-free, nitrile or similar surgical-quality gloves when handling sample containers.
- Never sample near a running vehicle. Do not park vehicles in immediate sample collection area (even non-running vehicles).
- Minimize the amount of time any sample container is left open.
- Do not set lids down where they may accumulate contaminants.
- Prevent foreign material (blowing dust, leaves, etc.) from entering any open sample container.
- Never touch the inside surfaces of sample bottles, lids, or composite carboys, even with gloved hands.
- Never touch the exposed end of a sampling tube.
- Avoid allowing rainwater to drip from rain gear into sample bottles.

- Do not eat or drink during sample collection.
- Do not breathe, sneeze or cough in the direction of an open sample bottle.

GRAB SAMPLE COLLECTION

Grab sampling may be required for certain toxicity studies (see ***Section 2***). Grab samples can be collected with or without the use of automated sampling equipment. Non-automated options include pumping the sample using a portable pump, direct submersion, or use of an intermediate container. When automated equipment is involved, the equipment can be paused to allow for grab sample collection. Each of these methods are discussed below.

Grab Sample Collection Without the Use of Automated Equipment

Grab samples can be collected by direct submersion of each individual sample container. In some instances it may not be possible to collect the sample directly into the sample container and an intermediate container will be required. When use of an intermediate container is appropriate, grab samples should be collected by holding an appropriate container (bailer, sample bottle, etc.) under the outfall of a discharge pipe, at the lip of an inlet grate, or by submerging a container in the discharge stream with the container opening facing upstream, depending on monitoring site configuration. The sample is then poured immediately into the appropriate grab sample bottle. Efforts should be made to collect samples without the use of intermediate containers. If unavoidable, ensure that intermediate containers are clean and are not used between sites (i.e. a clean container should be used at each sampling location where an intermediate container is necessary).

Grab samples also can be collected by pumping the sample into the appropriate sample container(s) using a portable peristaltic pump. First, clean tubing is installed into the pump. The intake end of the tubing is placed into the runoff stream at a point that is mid-stream and mid-depth. The output end of the tubing is held at the mouth of the appropriate sample container. Make sure that the tubing does not touch anything besides the sample container and the runoff. Completely fill sample containers so that there is no head space.

Grab Sample Collection Using Automated Equipment

At sites equipped with automated sampling equipment it is possible to use this equipment for grab sample collection. When automated equipment is programmed to collect composite samples and monitoring is in progress, sampling may be paused for grab sample collection. This is accomplished by pausing the sampler (see manufacturer's manual for instructions), and removing the pump tubing from the composite sample bottle using clean, powder-free gloves. Care should be used not to touch the end of the

tubing, even while wearing gloves. The tubing is then held at the mouth of the sample container(s) to be filled and the sampler is set to pump (see manufacturer's manual for instructions on starting the pump). Once sample container(s) are filled completely (leaving no head space), the tubing should be returned to its original position in the composite sample bottle and the sampler must be restarted (see manufacturer's manual) to return to automated composite sample collection. Clean handling techniques must be used when handling the pump tubing and sample bottle lids. When using this grab sample collection method, an effort should be made to collect the sample between collection of composite sample aliquots (i.e. immediately after the automated sampler has collected an aliquot). This will avoid or reduce disruption of composite sample aliquot collection timing.

COMPOSITE SAMPLE COLLECTION

A composite sample is made up of multiple sub-samples (aliquots) collected over some spatial or temporal range. Stormwater runoff composite samples are typically collected from a single location during a period of runoff. Such temporal composites can be collected on a time-proportioned basis (equal sample aliquot volumes collected at equal time intervals) or flow-proportioned basis (samples are collected either on an even-time-interval basis, with sample aliquot size proportional to instantaneous measured flow rate, or on an even-flow-interval basis, with a set aliquot volume collected at passage of each equal, pre-set flow volume). Because stormwater runoff flow typically varies throughout a storm event, flow-proportioned composite sampling is the standard composite sample collection method [see Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)] for guidance on the selection of sample collection methods and equipment). Composite samples are typically collected using automated sampling equipment, but can also be collected manually.

Flow-proportional sampling requires determination of several key parameters:

- Storm event quantity of precipitation forecast ("QPF"; from forecast information),
- Expected runoff volume (determined from the QPF and watershed characteristics),
- Expected storm duration (for even-time-interval methods),
- Minimum required composite sample volume for all planned toxicity tests (including TIEs) (see ***Section 9***) obtained from the contract toxicity laboratory,
- Minimum acceptable number of sample aliquots (see Table 7-1 and discussion below), and
- Sample aliquot size (varies proportional to measured flow throughout the event for even-time-interval methods, and set to a single volume per event for even-flow-volume methods)

See Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for a detailed discussion of how to determine flow-proportioning parameters.

Automated Composite Sample Collection

Automated flow-proportional composite sampling is typically done on an even flow-volume-per-sample basis; that is, a sample aliquot of equal size is collected every time a pre-selected flow volume passes by the flow sensor. The flow volume per sample is determined based on the QPF and the required composite sample volume [see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)], with consideration of the minimum required number of sample aliquots for the storm event (see Table 7-1 and discussion below). At automated monitoring stations, sample collection will begin automatically once the programmed thresholds (triggers) have been met (see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for automated station programming and preparation).

Automatic sampling stations should be checked periodically throughout a monitored storm event to make sure the station is functioning properly. If the composite sample bottle (or bottles, in the case of multi-bottle composite sample collection) fills more rapidly than expected, field personnel should be mobilized to conduct a bottle change (bottle changing is described in detail later in this section). If the required sample volume exceeds the capacity of one bottle, this will also require a bottle change. If the composite sample collection period exceeds 24 hours, the composite sample bottle(s) should be replaced with (a) clean bottle(s) at or prior to the end of each 24-hour period. Because the sample holding time is short for toxicity tests (36 hours), composite sample volume should be removed from each 24-hour composite for testing to comply with holding time requirements.

After the storm event has ended, field personnel are mobilized to retrieve the full composite sample bottle(s) and interrogate sampling equipment. Sample splitting and delivery to the laboratory are described later in this section.

Manual Composite Sample Collection

Manual composite sample collection may be conducted at monitoring sites that are not equipped with automatic equipment. Manual composite sample collection can be done on a time-proportional compositing basis, but it is generally possible to perform flow-proportional manual composite sampling using one of the techniques described below. Manual flow-proportional composite sample collection is conducted using the same basic principals as automatic composite sample collection. When possible, ensure that sample containers are completely filled, leaving no head space. Typical manual composite sample collection methods are described below.

Flow-Proportioning – Even Time Interval Basis

This method involves the collection of sample aliquots at a specified time interval, with the aliquot volume set proportional to the measured flow rate. The sampling time interval is set to ensure collection of the minimum number of aliquots required for adequate storm representativeness (see discussion later in this section), based on the expected (forecast) storm duration. Sample aliquot volumes are set to ensure collection of the required composite volume over the course of the expected storm event, based on the storm QPF and the expected runoff volume (this requires some advance knowledge of rainfall/runoff relationships in the monitored watersheds; see Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for a discussion of the relevant calculations). At each sampling interval the flow rate is measured [using one of the methods described in Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)] and a sample aliquot volume is collected in proportion to the measured flow prior to collection. Sample aliquots are composited to generate a single event composite sample. This method requires advance preparation of a table showing the aliquot volumes to collect for a range of expected flow rates, over a range of possible storm event QPFs. In this table, the aliquot volumes are set so as to ensure collection of the full composite sample volume required to perform all planned analyses for a given QPF.

Flow-Proportioning – Even Flow Volume Basis

This method is typically more difficult than the even-time-interval method described above, because it requires keeping a cumulative running tally of flow volume, which is not normally practical without automated flow monitoring equipment. As with automated composite sampling, flow volume per sample is calculated using the target storm QPF, with consideration for the required composite sample volume and the minimum acceptable number of aliquots, as discussed below (also see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a) for guidance on calculating flow volume per sample). Once runoff begins, flow rate is measured periodically [using one of the methods described in Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)], and sample aliquots are collected each time the pre-determined flow volume per sample has passed the sampling location. Instantaneous and cumulative flow volume can be calculated by inputting the flow data into a portable computer and applying Manning's equation or other appropriate flow equation in the field [see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)]. Sample aliquots are collected either as manual grabs or by using a peristaltic pump equipped with appropriate tubing and strainer [see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)]. Each sample aliquot collected is of equal volume (typically 250-1000 mL) and combined into a single composite bottle immediately after collection.

Flow-Proportioning Using Precipitation Measurement

For sites where flow measurement is extremely difficult (such as sites where sheet flow is prevalent) flow-proportional samples may be collected using precipitation measurements as an analog for runoff flow. The assumption is made that runoff volume is directly proportional to event precipitation. So, instead of calculating flow volume per sample, rainfall depth per sample is determined. A sample aliquot is then collected each time the selected precipitation increment has fallen. For this approach, an on-site rain gauge is required for precipitation measurement [see Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a)]. To determine appropriate rainfall amount per sample for a target storm event, simply divide the event QPF by the number of sample aliquots required (the number of sample aliquots required is determined by the total composite volume required and the desired sample aliquot volume, subject to the minimum numbers of sample aliquots per event, as discussed later in this section).

Multi-Bottle Flow-Proportioning

This method of flow-proportional sample collection involves collecting sample aliquots of equal volume at a predetermined time interval throughout the storm event, and recording the flow rate at the time of sample collection. The sample aliquot volume is set in advance, based on the expected duration of the storm and the required composite sample volume, and considering the minimum number of aliquots required (see discussion below). After the storm has ended, a portion of each aliquot is composited to generate a single flow-proportional composite sample. The volume used from each aliquot is directly proportional to the flow rate that was recorded during the aliquot collection. Using this method, the highest flow rate measured during the event is used to determine the scale for the sample volumes added to the composite from each aliquot. For example, for each aliquot, the flow reading taken during sample collection can be divided by the highest flow reading during the event, and that percentage is the percentage of the aliquot that is added to the composite. This method requires more bottles and bottle handling than the other methods described above. However, this method is commonly used due to its flexibility and relative ease in carrying out the procedures in the field.

FLOW MONITORING

Flow measurements are necessary to produce flow-weighted composite samples. Flow measurements should be performed utilizing one of the methods discussed in Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). If using manual methods, field crews should begin taking flow measurements as soon as possible after stormwater runoff begins (concurrently with sample collection). If automated sampling equipment is utilized, the equipment must be programmed to obtain the desired composite sample volume every time a specified flow volume is recorded, based on the predicted rainfall amount. Methods for programming automatic samplers

and flow meters are described in Part I, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).

If multiple bottles are used for composite sample collection, or if more flow volume is delivered than expected during a storm event, requiring one or more composite bottle changes per monitoring station, it is necessary to combine the multiple composite bottles to produce a single flow-weighted composite sample. To do this, it is normally necessary to use the collected flow data to determine the amount of sample from each composite bottle to be used to form the final composite. When using automated equipment, the field crew will typically download data from the flow meter or datalogger, to determine the flow volume represented by each composite bottle. The sample volumes to be used out of each composite bottle can be calculated by the monitoring crew, and the completed calculations faxed or otherwise delivered to the lab. The methods for calculating appropriate sample volumes from each bottle are described below under "Multi-bottle Compositing".

COMPOSITE BOTTLE CHANGING

If an automated monitoring station is used for the collection of composite stormwater samples and a composite bottle change is required, composite bottle changing is conducted using the steps listed below.

1. The automated sampling equipment is placed in pause mode prior to the initiation of a composite bottle change. This action is accomplished in the field or by remote monitoring personnel if the monitoring station is equipped with telemetry.
2. Field personnel should wear clean, powder-free nitrile gloves and practice clean sampling techniques (see above).
3. To change a sample bottle, the end of the pump tubing is removed from the full sample bottle, the full bottle is removed from the sampler and capped with a clean lid, a clean bottle is placed in the sampler, and the tubing end is placed into the clean bottle. Do not allow the exposed tubing end to contact hands or any other surface.
4. After the sample bottle has been changed, the sampler is closed and the sampler keypad is used to place the sampler in sampling mode. The field supervisor or remote operation personnel are notified as soon as the bottle change is complete.
5. The sampling team fills out the appropriate information on the label of the collected composite sample bottle(s).
6. The collected composite bottle(s) are surrounded with ice, and secured inside the vehicle for transport.
7. Verify that the automatic sampler has been placed in sampling mode, if sampling is to continue. Visually inspect the components for possible damage or clogging,

to be sure the system will be ready to continue sampling, or is ready to sample the next storm.

FIELD MEASUREMENTS

Field measurements, including pH, DO, temperature, and EC, should be taken at each site during sample collection (see ***Section 4***). Samples for field analysis should be collected using methods similar to grab sample collection. Collect samples by placing a sampling container directly into the runoff stream. Take measurements on the sample using appropriate meters, which should be calibrated according to manufacturer's standards prior to each targeted storm event, and record all measurements on the field log.

SAMPLE REPRESENTATIVENESS EVALUATION

Immediately following sample collection, composite sample representativeness must be evaluated to determine whether samples meet the project minimum acceptable storm capture parameters (number of aliquots and percent storm capture). Samples not meeting these criteria are generally not tested. However, the Caltrans Project Coordinator should be consulted to make the decision whether or not to analyze the samples.

Percent storm capture is the percentage of the total event flow that passes the sampling station during which sample collection occurred (i.e., the portion of the runoff represented by the composite sample). This is calculated simply by dividing the flow volume that passed the sampling station during sample collection by the total flow that passed the sampling station during the entire monitoring event.

The minimum acceptable number of sample aliquots and minimum acceptable storm percent capture depend on the total event precipitation, as shown in Table 7-1. The specified minimum number of sample aliquots is intended to ensure adequate representativeness of the composite sample throughout the monitoring event. Higher numbers of sample aliquots are desirable whenever possible, subject to the practical limitations of sample collection.

Table 7-1. Monitoring Event Representativeness Requirements

Total Event Precipitation	*Minimum Acceptable Number of Aliquots*	*Percent Capture Requirement*
0-0.25”	6	85
0.25-0.5”	8	80
0.5-1”	10	80
>1”	12	75

MULTI-BOTTLE COMPOSITING AND COMPOSITE SAMPLE SPLITTING

Procedures for combining multiple composite samples to produce a single sample, and the procedures for splitting samples into multiple sample bottles are described below.

Multi-bottle Compositing

When multiple composite sample bottles are filled at a single site during a single storm monitoring event, the sample bottles are typically composited together to produce a single composite sample representing the entire monitoring event.

To combine multiple sample bottles to generate a single representative composite sample, the following two items must be determined: 1) the percent of the sampling event flow represented by each individual sample bottle, and 2) which of the sample bottle(s), if any, will limit the compositing of samples. Because individual sample bottles will likely contain different volumes, one bottle will likely dictate the total available sample volume. Individual sample bottles may contain different sample volumes for several reasons. For example, the number of aliquots may differ in each bottle if runoff ceased before triggering all programmed sample aliquots. Composite bottle volumes may also differ due to unequal collection of aliquot volumes, sometimes caused by pump tubing blockages or wear.

Each individual composite sample bottle corresponds to the volume of stormwater runoff that passed the sampling point during the collection of that composite sample. Composite samples are mixed in relative proportion, according to the percentage of total volume that passed the sampling point during the storm event. Therefore, to properly combine multiple composite samples, the following must be known:

- Individual sample volumes in each sample bottle.
- Total runoff flow volume that passed during the collection of each individual sample bottle.
- The total runoff volume for the monitoring event.

Multiple composite samples should be combined using the following formulas:

$V_n/V_t = P_n$, and

$S_t * P_n = S_n$

Where;

V_n = the volume of flow that passed during the collection of bottle n
V_t = the total volume of flow that passed during the sample collection event
P_n = the percent of the total sampled flow represented by bottle n
S_t = the total volume of sample collected in all bottles combined
S_n = the volume of sample contributed from bottle n toward the combined composite sample

The following is an example of how multiple composite samples are combined:

Bottle #1 = 8 L of sample 10,000 CF passed during the collection of bottle #1 (V_1)

Bottle #2 = 10 L of sample 15,000 CF passed during the collection of bottle #2 (V_2)

Total volume passed during sample collection = 25,000 CF (V_t)

Total composite sample collected = 18 liters (S_t)

Bottle #1, % of total flow = % of composite = 10,000/25,000 = 40% (P_1)

Bottle #2, % of total flow = % of composite = 15,000/25,000 = 60% (P_2)

Therefore, the single composite is made up of 40% from bottle #1 and 60% from bottle #2. Since 40% of the total sample volume collected (18 liters) equals 7.2 (S_1) liters and 60% equals 10.8 liters (S_2), it is apparent that bottle #2 has limited sample volume for compositing. Therefore, the entire 10 liters of sample from bottle #2 is mixed with the following volume (X) from bottle #1.

10 liters/60% = X liters/40%

X (the volume required from bottle #1) = 6.7 liters

The two composite samples are combined by adding 6.7 liters from sample bottle #1 to 10 liters from sample bottle #2.

Each sample bottle must be well-mixed prior to pouring off into another composite bottle. The sample is mixed thoroughly by shaking or otherwise agitating the composite bottle to prevent sediment from remaining on the bottom of the bottle. Throughout the sample compositing procedures, clean, powder-free nitrile gloves are used for bottle and lid handling. This process can be done by analytical laboratory personnel, or by field sampling personnel in a clean, dry setting.

Composite Sample Splitting

Composite samples collected in a single composite sample bottle are poured, by the laboratory or the sampling team, into individual sample bottles for analysis (to limit contamination it is recommended that splitting be conducted by the laboratory). When toxicity testing is planned in conjunction with general stormwater monitoring, toxicity testing can be viewed by the sampling crew as an additional "constituent" for analysis. Therefore, samples for toxicity testing should be poured off into the appropriate sample containers in the same manner as samples for other analyses. When pouring off samples for toxicity testing, fill sample containers completely leaving no head space. When a composite sample duplicate is required, the sampling team will be required to split the composite sample into two composite bottles to generate a subsampling duplicate. As with field duplicate samples (replicate samples collected simultaneously in the field), subsampling duplicates (replicate samples generated from a single composite sample bottle) should be submitted to the analytical laboratory "blind" (labeled using a pseudonym site name). Below are three examples of composite sample splitting procedures:

<u>Sample Splitting Example #1</u>

1. Sample storage bottles are labeled for specific analyses.
2. Clean powder-free nitrile gloves are worn for handling of bottles and lids.
3. Any item that will contact the sample is cleaned using protocols presented in ***Appendix E*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).
4. Clean, specially blown borosilicate glass vessels are used to composite and mix samples (vessels have glass spigots with Teflon stopcocks).
5. Clean, Teflon coated magnetic mixing bars are used to stir the sample continuously before and during sample pour-off into individual sample containers (check with the toxicity laboratory for recommended sample volumes for toxicity tests).

<u>Sample Splitting Example #2</u>

1. Sample storage bottles are labeled for specific analyses.
2. Clean powder-free nitrile gloves are worn for handling of bottles and lids.
3. Any item that will contact the sample is cleaned using protocols presented in ***Appendix E*** in Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a).
4. During continuous manual composite sample agitation, sample is drawn from the bottle into individual sample containers using a portable peristaltic pump and clean tubing (check with the toxicity laboratory for recommended sample volumes for individual toxicity tests).

Sample Splitting Example #3 (USGS method)

1. Sample storage bottles are labeled for specific analyses.
2. Clean powder-free nitrile gloves are worn for handling of bottles and lids.
3. The composite sample is mixed thoroughly by carefully shaking composite bottle, with lid in place, until the sample is well mixed.
4. Immediately after mixing, the composite sample is poured into a clean Teflon funnel/splitter with clean tubes leading to individual sample containers (check with the toxicity laboratory for recommended sample volumes for toxicity tests).

Sample Splitting Example #4

1. Sample storage bottles are labeled for specific analyses.
2. Clean powder-free nitrile gloves are worn for handling of bottles and lids.
3. The composite sample is mixed thoroughly by carefully shaking composite bottle, with lid in place, until the sample is well mixed. For large composite bottles, the use of a swiveling mechanical bottle holding/mixing/pouring device is recommended.
4. Immediately after mixing, the composite sample is poured off into individual sample storage bottles and the bottles are capped (check with the toxicity laboratory for recommended sample volumes for toxicity tests). Step 3 is repeated immediately prior to filling each sample storage bottle.

SAMPLE PRESERVATION AND HOLDING TIME

Preservation

To minimize loss of toxicity due to volatilization of toxic constituents, all containers must be completely filled, leaving no head space between the contents and the lid. All samples are kept on ice or refrigerated to 4° Celsius from the time of sample collection until delivery to the analytical laboratory. Refrigerated automatic samplers are ideal for keeping composite samples cool during sample collection. Where refrigerated automatic samplers are not used, composite samples are kept on ice from the time sampling is initiated. Ice is checked regularly to insure that the sample is kept cool. Grab samples are placed in an ice chest with ice immediately following collection.

In addition to keeping stormwater samples cool it is also important to minimize the exposure of the samples to direct sunlight, as sunlight may cause chemical and or biological alteration of the sample, resulting in unreliable results. Use of opaque plastic cubitainers or amber glass bottles should be used to reduce light exposure and all samples should be covered or placed in an ice chest with a closed lid immediately following collection or removal from the automatic sampler enclosure.

Holding Time

It is imperative that all samples be delivered to the toxicity testing laboratory within 24 hours, and toxicity tests initiated within 36 hours. This is the acceptable holding time for freshwater acute and chronic toxicity tests. When more than one grab sample is taken or a composite sample is collected, sample holding time begins when the last grab sample in a series is taken, or when a 24-hr composite sampling period is completed. The Chain-of-custody (COC) form should clearly indicate the time of sample collection.

SAMPLE DELIVERY/CHAIN OF CUSTODY

All samples must be kept on ice, or refrigerated, from the time of onset of sample collection to the time of receipt by laboratory personnel. Composite samples should be kept on ice or refrigerated from the onset. If samples are being shipped to the laboratory, place sample bottles inside coolers with ice (wet ice is preferable to gel ice), ensure that the sample bottles are well packaged (i.e. with bubble wrap, foam, etc.), and secure cooler lids with packaging tape.

To minimize the risk of exceeding the 36 hour holding time, samples must be transferred to the toxicity laboratory as soon as possible after sampling, but must arrive at the toxicity laboratory no more than 24 hours after sample collection. The field crew must coordinate activities with the toxicity laboratory to ensure that the holding time can be met, especially for samples collected at night or on weekends. The sampling crew must contact the laboratory prior to sample collection to provide some indication as to when the laboratory should expect to receive the samples. This notice should be provided as soon as possible (i.e. during the pre-alert stage when a target storm is expected within the next 72 hours), and should be followed by notice to the laboratory once sample collection has commenced. This should allow the laboratory sufficient time to prepare for testing (e.g. obtain organisms, arrange for staff, etc.). For Caltrans toxicity studies, laboratories should preorder any required organisms in anticipation of stormwater samples and make arrangements to have staff available. Additionally, to limit the logistical issues (and associated costs), whenever possible, toxicity testing should be requested for samples anticipated from storms targeted between Monday through Friday. This will allow labs to commence testing during weekday hours.

COC forms must be filled out by the sampling team for all samples submitted to the toxicity laboratory. The purpose of COC forms is to keep a record of the transfer of sample custody, and requested analyses. Sample date, sample location, and analysis requested are noted on each COC, including specification of lab quality control requirements (e.g., laboratory duplicate samples; see ***Section 8***). Any special instructions for the laboratory should also be noted (e.g., request for follow-up TIEs). Customized project specific COCs, that include standard information (e.g., contact information, constituents and methods, and special notes) are recommended. Copies of COC forms are kept with field notes in a field log book. COC forms should be checked to be sure all analyses specified by the sampling plan are included. Review of the COC forms

immediately following a storm event gives the data reviewer a chance to review the field crews' requests and then to notify the laboratory of additional analyses or necessary clarification. An example of a customized COC form is presented as Figure 7-1.

Laboratory Name Laboratory Address

CHAIN-OF-CUSTODY RECORD **DATE:** **Lab ID:**

Client:	
Address:	
Sampled By:	
Phone:	
FAX:	
Project Manager:	
Project Number:	

Client Sample ID	**Sample Date**	**Sample Time**	**Sample Matrix**	**Container #**	**Container Type**	**REQUESTED ANALYSIS** Acute toxitiy *C. dubia*	Acute toxicity *P. promelas*										**Special Instructions/ Comments**
7-125			SW	1	1L glass	X											screening
10-128			SW	2	1L glass		X										dilution series

Correct Containers:	Yes	No		
Sample Temperature:	Ambient	Cold	Warm	
Sample Preservative:	Yes	No		
Turnaround Time:	Normal	Specify:	10/30 days	fax/final

Comments:

RELIQUINSHED BY		**RECEIVED BY**	
Signature:		Signature:	
Print:		Print:	
Company:		Company:	
Date:	Time:	Date:	Time:
Signature:		Signature:	
Print:		Print:	
Company:		Company:	
Date:	Time:	Date:	Time:
Signature:		Signature:	
Print:		Print:	
Company:		Company:	
Date:	Time:	Date:	Time:

Figure 7-1. Example Chain-of-Custody Form

SECTION 8
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)

The quality of toxicity data is dependent on the manner in which samples are collected, handled and analyzed. To ensure that data meet project data quality acceptability limits or objectives (DQOs,) a Data Quality Evaluation Plan (DQEP) should be included in the sampling and analysis plan (SAP) prior to implementing the toxicity study. The DQEP should include QA/QC review procedures for both field and laboratory measures (see ***Section 6*** and the QA/QC data evaluation discussion below).

This section covers the following topics:

- **Blank Samples**
- **Duplicate Samples**
- **QC Sample Schedule**
- **Lab-Initiated QA/QC**
- **QA/QC Data Evaluation**

For aquatic toxicity tests, the acceptability of test results is determined primarily by performance-based criteria for test organisms, culture and test conditions, and the results of control bioassays. Control bioassays include testing with reference toxicants and negative controls (dilution water). Test acceptability requirements are documented in the method documents for each bioassay method.

QA practices for toxicity testing methods must address all factors that affect the quality of the final toxicity test data, such as:

- Sample collection and handling
- The source and condition of the test organisms
- Condition of equipment
- Test conditions
- Instrument calibration
- Replication
- Use of reference toxicants

In addition to the QA requirements for toxicity testing methods, a designated percentage of samples collected for toxicity studies should be reserved for QC analyses. These analyses should consist of laboratory duplicates and field blanks. This section discusses the QA/QC samples, as listed below, that should be included in toxicity studies. Additionally, a QC schedule should be developed to outline the types and frequency of QC samples to be conducted during a given toxicity study. Finally, data should be

evaluated to determine whether the project's data quality acceptability limits or objectives (DQOs) have been met.

BLANK SAMPLES

Blanks are prepared to identify potential sample contamination occurring during field collection, handling, shipment, storage, and laboratory handling and analysis. Blanks are prepared during various stages of the sampling and analytical process to determine the level of contamination, if any, introduced at each step. The collection and uses of the types of blank samples associated with typical stormwater monitoring field procedures and toxicity test methods are described below. "Blank water" for toxicity studies refers to the standard dilution water[1] that is prepared and utilized by the toxicity laboratory performing the environmental and blank analyses. Laboratory dilution water is also used for test controls.

Equipment Blanks

Before using sampling equipment for environmental sample collection, blanks should be collected to verify that the equipment is not a source of sample contamination. Stormwater monitoring programs normally include equipment blanks to check sample tubing and strainers, and sample bottles. Equipment blanks are usually prepared at the beginning of the monitoring season, and are collected by passing blank water through clean equipment. Equipment blank samples are collected using clean techniques, prior to on-site sample collection (before the equipment has been contaminated by environmental sample water or other sources). Equipment blank collection normally requires a two-person field crew. After collection, equipment blanks are capped, placed in an ice chest with ice and delivered to the analytical laboratory in the same manner as environmental samples.

If using manual collection methods (i.e. bailers), fill the bailer or other collection device with blank water in the field and pour the water into clean sample bottle(s). If an automatic sampler is being used, place the cleaned strainer on the end of the clean tubing, and install the tubing through the sampler. Do not install the tubing and strainer into the pipe or channel, but leave the tubing coiled up beside the sampler. Prevent the strainer or exposed tubing end from contacting anything other than the inside of a new plastic bag during this process. Then insert the strainer into the bottle containing the blank water and pump the sample through the clean suction tubing, into clean composite bottle(s) and/or grab sample containers.

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[1] A synthetic (standard) dilution water (moderately hard water) is normally used for the types of tests outlined in this guidance document. References to blank water in this section refers to toxicity laboratory dilution water.

Equipment blanks are analyzed using the same methods as those used for normal sample analysis and submitted to the toxicity laboratory "blind" (disguised as a regular environmental sample by labeling with a pseudonym).

Field Blanks

Field blanks are necessary to evaluate whether contamination is introduced during field sampling activities. Field blanks are prepared by the field crew, under normal sample collection conditions, at some time during the collection of normal stormwater samples. Field blanks are prepared by filling a large carboy or other appropriate container with blank water, transporting the container to the field and processing the water through the same sampling procedures to be used for sample collection. If grab sample collection is conducted, grab sample field blanks should be prepared by pouring a sample directly from the bottle of blank water, into the grab sample containers. Grab sample blanks should imitate environmental sampling as closely as possible by using grab poles, clean intermediate containers, and other clean equipment in the same manner. For composite field blanks, pour the blank water directly into clean composite bottles at the sampling site, label as an environmental sample, and submit “blind” to the laboratory. The filled blank sample bottles should be sealed and sent to the laboratory to be analyzed for the required constituents.

In addition to the minimum field blank collection requirements indicated in Table 8-1, it is important that field blanks are collected at a frequency no less than once per field sampling team per sampling season. Additional blanks should be collected when there is a change in sampling personnel, equipment, or procedures. It may also be desirable to prepare field blanks prior to any actual sampling events as an advance check of the overall sampling procedures.

DUPLICATE SAMPLES

Laboratory duplicates are used to assess the precision of the toxicity testing method and laboratory handling (intra-laboratory variability). For laboratory duplicate samples, one sample will be split by the toxicity laboratory into two portions and each tested. When collecting samples to be analyzed for laboratory duplicates, the normal sample volume required should typically be doubled. This requires filling a larger size sample bottle (preferred), or filling two normal size sample bottles.

QC SAMPLE SCHEDULE

Table 8-1 summarizes the recommended frequencies of QC sample collection/ preparation for Caltrans toxicity studies. These frequencies are minimum and may be increased depending on the nature and objectives of the study being undertaken or if QA/QC problems (e.g. contamination) are discovered. An adequate QC sample schedule should be included in the analytical budget for the toxicity study.

A QC sample schedule should be developed, included in the SAP, and followed closely by field personnel. The project QC sample schedule should meet the minimum QC sample frequency criteria over the term of the project. In some cases, QC samples at one site may be applied to an analogous situation at another site. For example, if the same equipment and protocols are used at Site A and Site B, equipment blank checks at one site can be applied at both sites. The minimum frequency criteria must still be met; however, the QC sample schedule should consider the location of each QC sample to maximize coverage at all project sites. A QC sample schedule should, at a minimum, indicate the types and frequency of QC sample collection, site of QC sample collection, and QC sample pseudonyms for samples that will be submitted "blind" to the laboratory.

Table 8-1. Recommended QC Sample Frequency

QA/QC Sample Type	***Minimum Sampling Frequency***
Equipment Blank	Equipment blanks should be collected prior to each sampling season for each sampling method to be utilized (e.g. automated, manual).
Field Blank	One field blank should be collected per storm event/batch of samples. The site at which the field blank is collected should be rotated throughout the course of the study.
Laboratory Duplicate	Ten percent of the samples collected for aquatic toxicity testing should be split and tested in duplicate by the toxicity laboratory.
Reference Toxicants (see following section)	Toxicity laboratories must run a reference toxicant test monthly.

1 A batch is defined as the group of bottles that have been cleaned at the same time, in the same manner; or, if certified bottles are sent directly from the manufacturer, the batch would be the lot designated by the manufacturer in their testing of the bottles.

LAB-INITIATED QA/QC

QA/QC measures initiated by the toxicity laboratory include toxicity controls and reference toxicants.

Toxicity Controls

For aquatic toxicity testing, a control consisting of dilution water is run with every test. These toxicity controls are run side by side with environmental samples tested during each toxicity test conducted by the laboratory. Control treatments (organisms in unaltered dilution water) provide an important reference for dilution water and organism acceptability. Results of toxicity controls are used to determine the acceptability and significance of toxicity test results (see test acceptability criteria discussion in this section).

Reference Toxicants

Toxicity laboratories must conduct reference toxicant tests for each type of test to be used in the laboratory. For a given test method, successive tests must be performed with the same reference toxicant, at the same concentrations, in the same dilution water, using the same data analysis methods. According to U.S. EPA guidance (U.S. EPA, 1993a, 1994), the laboratory must conduct reference toxicant tests at a frequency no less than once per month. ***However, when possible, reference toxicant tests should be conducted when Caltrans samples are being tested in-house***.

QA/QC DATA EVALUATION

All data reported by the analytical laboratory must be carefully reviewed to determine whether the project's data quality acceptability limits or objectives (DQOs) have been met. The data review methods should be outlined in a DQEP developed for the project. A detailed discussion of the QA/QC data evaluation process for Caltrans stormwater monitoring programs is presented in Part II, Caltrans Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). This process includes two steps; initial data quality screening and data quality evaluation.

Data quality screening includes the following:

1. Verification check between sampling and analysis plan (SAP), chain of custody forms, and laboratory data reports;
2. Check of laboratory data report completeness; and
3. Check for typographical errors and apparent incongruities.

See the Caltrans Monitoring Protocol Guidance Manual: Water Quality (Caltrans, 2000a) for details concerning the above.

The data quality evaluation process for toxicity data is significantly different from the process developed for water chemistry. Data evaluation as it relates to toxicity testing includes review of the following QA/QC parameters:

- Holding times
- Test acceptability criteria
- Contamination check results (field, bottle, and equipment blanks)
- Precision and accuracy analysis (laboratory duplicates, reference toxicants)

Evaluation of each of these components is discussed below. Guidelines for evaluating these QA/QC elements are presented in Figure 8-1.

Holding Times

> ***Holding Times***
>
> Delivery to lab = within 24 hours
>
> Test initiation = within 36 hours

Holding time represents the period of time between sample collection and initiation of the toxicity test(s). Calculate the time elapsed between sampling and start of toxicity test initiation, and compare this to the required holding time. For composite samples that are collected within 24-hours or less, the time of the final sample aliquot is considered the "sample collection time" for determining sample holding time. Because toxicity test methods have a critical holding time (≤36 hours), sampling crews must take into account the time required to transport the sample to the laboratory. Sampling crews must notify the toxicity laboratory prior to and upon commencment of sample collection; and samples must arrive at the toxicity laboratory no later than 24 hours after collection. The COC form should clearly indicate the time of sample collection. If sampling crews fail to contact the laboratory prior to sample collection and/or samples older than 24 hours are received by the laboratory, the laboratory will not be held accountable for missed holding times. If the 36 hour holding time is exceeded, the laboratory should discard the samples.

Test Acceptability Criteria

Test acceptability criteria are used to demonstrate the sensitivity of the test organisms and the laboratory performance with a routine reference toxicant. Data acceptability criteria for each acute and chronic toxicity method (as outlined in ***Section 4***) are presented in Table 8-2 . If the test acceptability criteria are not met, the acceptability of the test will depend on the best professional judgment of the laboratory investigator. Any failure to meet the criteria must be noted when reporting data from a test. Efforts should be taken by the laboratory to resolve potential problems made evident by the test results.

In addition to acceptable criteria for control organisms, U.S. EPA toxicity test methods outline acceptable ranges for test conditions such as DO, pH, and temperature. An individual test may be conditionally acceptable if these specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests. The acceptability of the test will depend on the experience and professional judgement of the laboratory analyst and/or the reviewing staff of the regulatory authority. Any deviation from test specifications must be noted by the laboratory when test data are reported (U.S. EPA, 1993a, 1994).

Table 8-2. Aquatic Toxicity Test Acceptability Criteria

Test Method	Acceptability Criteria
Acute	
Ceriodaphnia dubia	90% or greater survival in controls
Pimephales promelas	90% or greater survival in controls
Oncorynchus mykiss	90% or greater survival in controls
Chronic	
Ceriodaphnia dubia	80% or greater survival and an average of 15 or more young per surviving female in the control solutions. 60% of surviving control organisms must produce three broods.
Pimephales promelas	80% survival in the controls, average dry weight per surviving organism in control chambers equals or exceeds 0.25 mg.
Selenastrum capricornutum	1 x 10^6 cells/mL with EDTA or 2 x 10^5 cells/mL without EDTA in the controls, variability of controls should not exceed 20%.

Contamination

Blank samples are used to identify the presence and potential source of sample contamination and as mentioned above typically include field blanks, bottle blanks, and equipment blanks. If toxicity is observed in blanks a complete review of field procedures and sample handling should be conducted as well as a detailed review of raw laboratory data and procedures. Often times it is necessary to refer to historical equipment blank results, if available, corresponding data (e.g. controls, reference toxicant tests), and field notes to identify contamination sources. This is a corrective and documentative step in the QA/QC process that should be conducted as soon as toxicity is reported for any blanks.

Precision and Accuracy

Reference Toxicant Tests

It is a laboratory's responsibility to demonstrate its ability to obtain consistent and accurate results with appropriate reference toxicants before it performs toxicity tests with environmental samples. To meet this requirement, the intralaboratory precision, expressed as percent coefficient of variation (CV%), of each type of test to be used in the laboratory, should be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions (i.e. duration, type of dilution water, age of test organisms, feeding, etc.), and same data analysis methods. These reference toxicant tests assess the accuracy and precision in the same way that repeated analysis of standard reference materials (SRM) provides both accuracy and precision for chemical analyses. A reference toxicant concentration series (0.5 or higher) should be selected that will consistently provide partial mortalities at two or more concentrations.

Toxicity endpoints from the most recent 25 reference toxicant tests should be used to construct control limits. The control limits consist of two standard deviations above and below the concentration representing the central tendency. An example control chart is illustrated in Figure 8-2. Outliers, which fall outside of the upper and lower limits, and trends of increasing or decreasing sensitivity, are readily indentified using this method. Control limits of $\pm$ 2 standard deviations will be exceeded 5% of the time by chance alone, regardless of how well a laboratory performs (U.S. EPA, 1993, 1994). Additionally, highly proficient laboratories which develop very narrow control limits may be unfairly penalized if a test result which falls just outside the control limits is rejected *de facto*. For this reason, the width of the control limits should be considered when determining whether the outliers should be rejected (U.S. EPA, 1995). Laboratories should consult U.S. EPA (2000) additional information on method variability in toxicity applications.

If the toxicity value from a given test with a reference toxicant falls well outside the expected range for the other test organisms when using the standard dilution water and test conditions, the sensitivity of the organisms and the overall credibility of the test should be considered as provisional and subject to careful review. In this case, the test procedure should be examined for defects and efforts should be made to resolve the problem.

Laboratory Duplicates

Laboratory duplicates are used to assess the precision of the toxicity method and laboratory handling. Duplicates should not be significantly different at the 5% level of significance, acceptability of the test will be depend on the best professional judgment of the laboratory investigator through careful review of results.

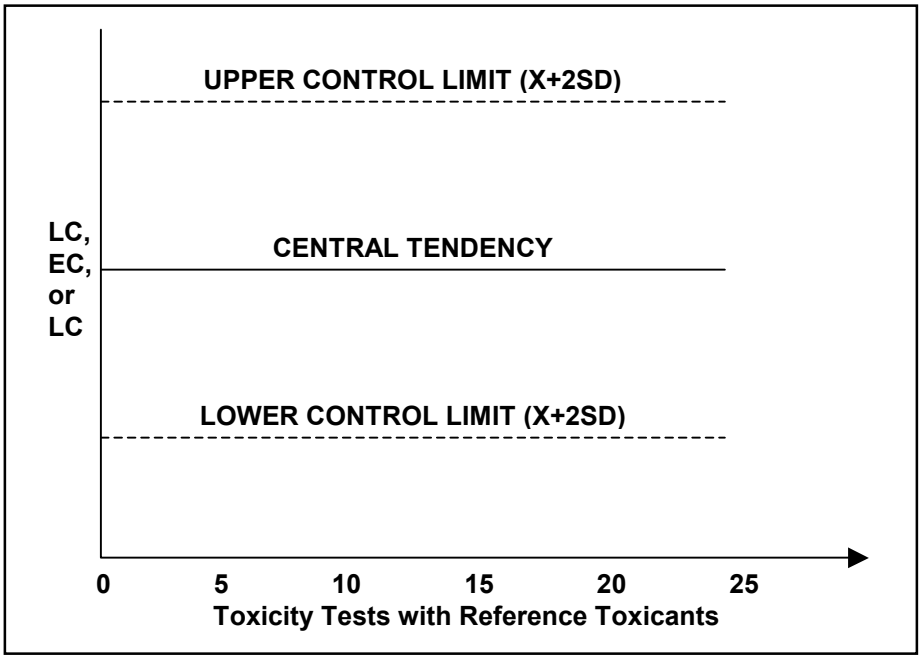

Figure 8-2. Example Reference Toxicant Control Chart

SECTION 9
LABORATORY CONTRACTING AND PROCEDURES

This section describes considerations involved in selecting and contracting with toxicity laboratories, the steps to be taken by toxicity laboratories to prepare for monitoring events, and the procedures laboratories will use for toxicity testing of stormwater samples. The following topics are discussed:

- **Laboratory Selection and Contracting**
- **Pre-Sampling Preparations**
- **Sample Storage and Handling Prior to Analysis**
- **Toxicity Test Methods**
- **Laboratory Data Package Deliverables**

LABORATORY SELECTION AND CONTRACTING

Important considerations in selecting a toxicity laboratory include performance, ability to meet test acceptability criteria, and experience with the types of toxicity testing included in the study. The laboratory should have a proven record of performance with the sample matrices involved in the study and the particular types of toxicity tests to be conducted for the project. Additionally, Department of Health Services (DHS) certification is required for Caltrans analytical and toxicity testing work.

It is important to ensure that the selected toxicity laboratory has proven satisfactory performance. The ability of the laboratory to obtain consistent, precise results must be demonstrated with reference toxicants. Reference toxicant data should be checked for values outside of the upper and lower control limits (see ***Section 8***). Data from laboratory controls should also be checked to ensure that the laboratory can consistently meet the test acceptability criteria established for controls.

Finally, it is necessary that laboratories be made contractually accountable to meet all Caltrans' data quality acceptability limits or objectives (DQOs) and project specifications. This is discussed in more detail later in this section.

PRE-SAMPLING PREPARATIONS

The laboratory will be involved in a number of activities prior to the actual testing of stormwater samples, including:

- Determination of key laboratory performance requirements (e.g., turnaround times, report formats) for toxicity testing services contract.

- Review and comment on the SAP and the data quality evaluation plan (DQEP), QC sample schedule, and QC sample volumes. This overall package is sometimes referred to as the QA/QC plan.
- Approach for conducting TIEs, if included in the study.
- Providing sampling team with clean sample containers and blank water.
- Coordination with sampling team prior to each anticipated storm sampling event.

These items are further discussed below.

Determination of Laboratory Performance Criteria

The toxicity laboratory must test the stormwater samples using methods that will achieve Caltrans' DQOs for the project (see ***Section 2***). The contract for toxicity testing services should specify laboratory performance criteria designed to ensure that the project DQOs will be met. The contract should specify the following:

- Test acceptability criteria
- Holding times
- Types and frequency of QA/QC analyses to be performed
- Quality control performance guidelines
- Sample turnaround times
- Electronic and hard copy report formats
- Corrective action procedures

In addition, the contract with the laboratory should specify that at least 90% of the sample results must meet the QA/QC criteria and be deemed usable for the project.

Laboratory Input to Project QA/QC Plan

The contract laboratory should review and provide input on the SAP and the QA/QC plan for each Caltrans toxicity study. This input will help ensure that the correct sample containers, sample volumes, holding times, and testing methods are specified, as well as the correct points of contact for communications between field and laboratory personnel. In addition, the laboratory should be involved early in the process so they can provide feedback on methods and performance standards during the planning phase.

Sample Containers, Blank Water

As discussed in ***Section 7***, sampling personnel will need to request the appropriate containers from the laboratory prior to each sampling event. The containers needed for a given project will vary depending on the toxicity test methods (i.e. if TIEs are planned) sample collection procedure, and the DQOs. For example, stormwater monitoring programs often include composite samples, wherein subsamples collected during a storm

event may be combined in one or more large containers. After the composite sample has been delivered to the laboratory, water is transferred from the composite sample containers into the appropriate containers. U.S. EPA methods for acute and chronic toxicity testing (U.S. EPA 1993, 1994) recommend the use of 4-liter (1 gallon) cubitainers for sample containment. However, U.S. EPA TIE methods (U.S. EPA, 1991) state that glass containers are preferable to plastic when TIE issues are of concern (see bottle selection discussion in ***Section 7***). As noted above, the toxicity laboratory should review the QA/QC plan for each Caltrans toxicity study to ensure that the plan specifies the appropriate sample containers and volumes.

If a given project calls for collection of composite samples, the field personnel must obtain the appropriate composite sample containers from the laboratory; however, the field personnel do not need the individual containers required for toxicity sample containment if the lab will be doing the composite sample breakdown. The composite sample bottle container must be large enough to provide sufficient volume of sample for all of the planned tests. Bottle contamination issues should always be assessed with blank samples, regardless of the bottle material (see ***Section 8***).

For toxicity studies that specify collection of grab samples, field personnel must obtain the appropriate pre-cleaned bottles from the laboratory prior to the sampling event. Bottles should be rinsed prior to sample collection according to the instructions presented in ***Section 7***.

Recommended sample volumes for acute and chronic toxicity tests are presented in Table 9-1. However, sample volumes should be confirmed with the laboratory prior to sample collection, including sufficient sample volumes for TIE follow-up testing (if this is planned) and required laboratory QA/QC analysis. As discussed in ***Section 8***, laboratory QC samples that make use of sample water provided by the field crew include laboratory duplicates. Duplicates typically require double the normal sample volume required.

If field blanks are to be collected, the laboratory also will need to provide sufficient quantities of blank water and appropriate containers (see ***Section 8***). The blank water supplied by the laboratory should be the same as the water used for dilution and toxicity controls.

Laboratory Coordination Prior to a Sampling Event

It is important for the sampling task manager to notify the laboratory of an anticipated storm sampling event so that the laboratory can prepare for possible off-hour sample delivery, and to make preparations to meet the 36 hour holding time for toxicity test initiation. The laboratory contact should be notified regarding the number of samples anticipated, approximate date and time of sampling (if known), and when sample containers, blank water or ice chests will be required.

In addition, the laboratory should be made aware of specific project requirements such as required laboratory performance objectives, required QC samples, and reporting requirements.

Table 9-1. Toxicity Method and Sample Specifications

Test Species	U.S. EPA Method Reference	Test Duration	Container Type [1]	Sample Volume Required [2]	Holding Time	Storage
Acute						
Ceriodaphnia dubia	EPA/600/ 4-90/027	24, 48, or 96 hrs	PE or glass	1 L	36 hrs	4° C
Pimephales promelas	EPA/600/ 4-90/027	24, 48, or 96 hrs	PE or glass	2 L	36 hrs	4° C
Oncorhynchus mykiss	EPA/600/ 4-90/027	24, 48, or 96 hrs	PE or glass	20 L	36 hrs	4° C
Chronic						
Ceriodaphnia dubia	EPA/600/4-91/002 (method 1002.0)	Max. 8 days [3]	PE or glass	7 L (1.0 L/day)	36 hrs	4° C
Pimephales promelas	EPA/600/4-91/002 (method 1000.0)	7 days	PE or glass	17.5 L (2.5 L/day)	36 hrs	4° C
Selenastrum capricornutum [5]	EPA/600/4-91/002 (method 1003.0)	96 hrs	PE or glass	1 or 2 L [4]	36 hrs	4° C

[1] If TIE follow-up tests are planned, samples should be collected in glass containers.
[2] Volume requirements are minima additional volume is needed if TIE follow-up is planned.
[3] Test is conducted until 60% of surviving control organisms have three broods.
[4] Is dependant upon test solution volume selected. This should be verified with the toxicity laboratory prior to sample collection.
[5] Species name has been changed to *Raphidocelis subcapitata.*

SAMPLE STORAGE AND HANDLING PRIOR TO ANALYSIS

To minimize the chance of sample contamination and unreliable results, special measures must be taken during the storage and handling of samples prior to analysis. For example, toxicity samples must be collected and stored in the appropriate containers at 4°C. If composite samples are collected, sample splitting must be conducted to properly store the samples. In addition, some toxicity test methods require filtration of the sample prior to test initiation. Finally, samples must be analyzed within established holding times to ensure reliability of the results. Each of these measures is discussed in more detail below and summarized in Table 9-1 for specific toxicity test methods.

Composite Splitting

If composite sampling procedures are used, the composite samples may need to be split prior to toxicity test initiation. It is recommended that composite sample splitting be conducted by the laboratory or in another similarly controlled environment to minimize the chance of contamination. Clean techniques should be used when handling and splitting the composite sample. Sample splitting procedures are explained in detail in ***Section 7***.

Sample Filtration

For toxicity tests utilizing *Ceriodaphnia dubia* or *Pimephales promelas* (fathead minnows), samples must be filtered through a 60 micrometer (μm) plankton net to remove indigenous organisms that may attack or be confused with the test organisms. Samples should be filtered in the laboratory as required for specific toxicity tests.

Sample Handling and Preservation

To minimize the loss of toxicity due to volatilization, all sample containers should be completely filled, leaving no air space between the contents and the lid. Samples should be chilled and maintained at $4^{o}C$ until used for toxicity testing to inhibit microbial degradation, chemical transformations, and loss of highly volatile toxic substances.

Holding Times

Every effort must be made to initiate toxicity tests with a stormwater sample on the day of arrival in the laboratory, and the sample holding time should not exceed 36 hours (see ***Section 8***).

TOXICITY TEST METHODS

Test methods should be selected that are sufficient to meet the DQOs for each project (***Section 2*** describes how project DQOs are developed). As discussed in ***Section 4***, toxicity test methods are selected based on regulatory and legal requirements, historical data, BMP goals, and other considerations.

In selecting the toxicity test method(s) to be used, the following questions should be addressed:

- Does the method conform to any legal or regulatory requirements for the monitoring program?
- Is the method appropriate for stormwater samples (has the laboratory successfully performed the method using stormwater samples)?
- Will the data provided by the method be comparable to historical data collected at the station?

- Is the method recognized as "standard" so that the data collected at a station can be compared to other stations?
- Is the laboratory proficient with the method? Do they have historical data to show proficiency?

As indicated in ***Section 4***, the toxicity test types recommend for use in Caltrans toxicity studies include freshwater acute testing using *Ceriodaphnia dubia*, *Pimephales promelas*, and *Oncorhynchus mykiss*; and chronic testing using *Ceriodaphnia dubia, Pimephales promelas,* and *Selenastrum capricornutum*. Summaries of the testing protocols for each of these tests are presented in Tables 9-2 through 9-7.

LABORATORY DATA PACKAGE DELIVERABLES

As a part of the laboratory contract, the data package that will be delivered to Caltrans and the timing of its delivery (turn around time) should be defined. The data package should include a narrative that outlines any problems, corrections, anomalies, and conclusions, as well as completed chain of custody documentation. A list of the minimum reporting parameters that should be included in the toxicity laboratory's report for each toxicity test is presented in Figure 9-1. The laboratory should include specifics concerning QA/QC results including the following:

- Adherence to holding times
- Any violations of test acceptability criteria
- Interpretation of field, bottle, and equipment blanks results
- Interpretation of laboratory duplicates, reference toxicants

Common turn around times for laboratory data packages three weeks to thirty days for the hard copy. The hard copy data package and any electronically supplied data should undergo data review as described in ***Section 8***.

Table 9-2. Summary of Test Conditions for *Ceriodaphnia dubia* Acute Toxicity Tests

Test type:	Static non-renewal or static renewal
Test duration:	24, 48, or 96 h
Temperature:	20 ± 1^0 C; or 25 ± 1^0 C
Light quality:	Ambient laboratory illumination
Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c) (ambient laboratory levels)
Photoperiod:	16 h light, 8 h darkness
Test chamber size:	30 mL (minimum)
Test solution volume:	15 mL (minimum)
Renewal of test solutions:	Minimum, after 48 h
Age of test organisms:	Less than 24-h old
No. organisms per test chamber:	Minimum, 5
No. replicate chambers per concentration:	Minimum, 4
No. organisms per concentration:	Minimum, 20
Feeding regime:	Feed YCT[1] and *Selenastrum* while holding prior to test; newly-released young should have food available a minimum of 2 h prior to use in a test; add 0.1 mL each of YCT and Selenastrum 2 h prior to test solution renewal at 48 h
Test chamber cleaning:	Not required
Dilution water:	Moderately hard synthetic water
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Mortality (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	90% or greater survival in controls

1 YCT = a mixture of yeast, digested trout chow, and cereal leaves (dried and powdered).

Table 9-3. Summary of Test Conditions for Fathead minnow, *Pimephales promelas* Acute Toxicity Tests

Test type:	Static non-renewal or static renewal
Test duration:	24, 48, or 96 h
Temperature:	20 ± 1^0 C; or 25 ± 1^0 C
Light quality:	Ambient laboratory illumination
Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c) (ambient laboratory levels)
Photoperiod:	16 h light, 8 h darkness
Test chamber size:	250 mL (minimum)
Test solution volume:	200 mL (minimum)
Renewal of test solutions:	Minimum, after 48 h
Age of test organisms:	1-14 days; 24-h range in age
No. organisms per test chamber:	Minimum, 10
No. replicate chambers per concentration:	Minimum, 2
No. organisms per concentration:	Minimum, 20
Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate 2 h prior to test solution renewal at 48 h
Test chamber cleaning:	Not required
Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
Dilution water:	Moderately hard synthetic water
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Mortality (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	90% or greater survival in controls

Table 9-4. Summary of Test Conditions for Rainbow Trout, *Oncorhynchus mykiss* Acute Toxicity Tests

Test type:	Static non-renewal or static renewal
Test duration:	24, 48, or 96 h
Temperature:	12 ± 1^0 C
Light quality:	Ambient laboratory illumination
Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c) (ambient laboratory levels)
Photoperiod:	16 h light, 8 h darkness. Light intensity should be raised gradually over a 15 minute period at the beginning of the photoperiod, and lowered gradually at the end of the photoperiod, using a dimmer switch or other suitable device.
Test chamber size:	5 L (minimum)
Test solution volume:	4 L (minimum)
Renewal of test solutions:	Minimum, after 48 h
Age of test organisms:	15-30 days (after yolk sac absorption to 30 days)
No. organisms per test chamber:	Minimum, 10
No. replicate chambers per concentration:	Minimum, 2
No. organisms per concentration:	Minimum, 20
Feeding regime:	Feeding not required
Test chamber cleaning:	Cleaning not required
Test solution aeration:	None, unless DO concentration falls below 6.0 mg/L; rate should not exceed 100 bubbles/min
Dilution water:	Moderately hard synthetic water
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Mortality (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	90% or greater survival in controls

Table 9-5. Summary of Test Conditions for *Ceriodaphnia dubia* Chronic Toxicity Tests

Test type:	Static renewal
Temperature:	25 ± 1^0 C
Test duration:	Until 60% of surviving control organisms have three broods (maximum test duration 8 days)
Light quality:	Ambient laboratory illumination
Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c) (ambient laboratory levels)
Photoperiod:	16 h light, 8 h darkness
Test chamber size:	30 mL (minimum)
Test solution volume:	15 mL (minimum)
Renewal of test solutions:	Daily
Age of test organisms:	Less than 24-h old; all released within a 8-h period
No. neonates per test chamber:	1
No. replicate chambers per concentration:	10
No. neonates per concentration:	10
Feeding regime:	Feed 0.1 mL each of YCT[1] and algal suspension per test chamber daily
Cleaning:	Use freshly cleaned glass beakers or new plastic cups daily
Aeration:	None
Dilution water:	Moderately hard synthetic water
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Survival and reproduction (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	80% or greater survival and 15 or more young per surviving female in the control solutions. 60% of surviving control organisms must produce three broods.

1 YCT = a mixture of yeast, digested trout chow, and cereal leaves (dried and powdered).

Table 9-6. Summary of Test Conditions for Fathead minnow, *Pimephales promelas* Chronic Toxicity Tests

Test type:	Static renewal
Test duration:	7 days
Temperature:	25 ± 1^0 C
Light quality:	Ambient laboratory illumination
Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c) (ambient laboratory levels)
Photoperiod:	16 h light, 8 h darkness
Test chamber size:	500 mL (minimum)
Test solution volume:	250 mL (minimum)
Renewal of test solutions:	Daily
Age of test organisms:	Newly hatched larvae less than 24 h old. If shipped, not more than 48 h old, 24 h range in age
No. larvae per test chamber:	15 (minimum of 10)
No. replicate chambers per concentration:	4 (minimum 3)
No. larvae per concentration:	60 (minimum 30)
Source of food:	Newly hatched *Artemia* nauplii (less than 24-h old)
Feeding regime:	Feed 0.1 g newly hatched (less than 24-h old) brine shrimp nauplii three times daily at 4-h intervals or, as a minimum, 0.15 g twice daily, 6 h between feedings. Sufficient nauplii are added to provide an excess. Larvae fish are not fed during the final 12 h of the test.
Test chamber cleaning:	Siphon daily, immediately before test solution renewal
Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
Dilution water:	Moderately hard synthetic water
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Survival and growth (weight) (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	80% or greater survival in controls; average dry weight per surviving organism in control chambers equals or exceeds 0.25 mg

Table 9-7. Summary of Test Conditions for Alga, *Selenastrum capricornutum* Chronic Toxicity Tests

Test type:	Static non-renewal
Test duration:	96 h
Temperature:	25 ± 1^0 C
Light quality:	"Cool white" fluorescent lighting
Light intensity:	86 ± 8.6 µE/m^2/s (400 ± 40 ft-c or 4306 lux) (ambient laboratory levels)
Photoperiod:	Continuous illumination
Test chamber size:	125 mL or 250 mL[1]
Test solution volume:	50 mL or 100 mL[1]
Renewal of test solutions:	None
Age of test organisms:	4 to 7 days
Initial cell density in test chambers:	10,000 cells/mL
No. replicate chambers per concentration:	4 (minimum 3)
Shaking rate:	100 cpm continuous
Test solution aeration:	None
Dilution water:	Algal stock culture medium, enriched uncontaminated source of receiving water, synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals, or DMW (see U.S. EPA 1994)
Test concentrations:	Screening level tests – 100% and control Dilution series – to be determined depending on the objectives of the study
Endpoint:	Growth (cell counts, chlorophyll fluorescence, absorbance, biomass) (see ***Section 10*** for more information)
Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period (see ***Section 8***)
Test acceptability criterion:	1×10^6 cells/mL with EDTA or 2×10^5 cells/mL without EDTA in the controls: Variability of controls should not exceed 20%

1 For tests not continuously shaken use 25 mL in 125 mL flasks and 50 mL in 250 mL flasks.

SAMPLE INFORMATION
1. Identify source water (field sample id, laboratory id) 2. Contract and task order numbers 3. Chain of custody
TEST METHODS
1. Toxicity test method used (title, number, source) 2. Endpoint(s) of test 3. Deviation(s) from reference method, if any, and the reason(s) 4. Date and time test started 5. Data and time test terminated 6. Type and volume of test chamber 7. Volume of solution used per chamber 8. Number of organisms per test chamber 9. Number of replicate test chambers per treatment 10. Acclimation of test organisms (temperature mean and range) 11. Test temperature (mean and range) 12. Specify if aeration was needed 13. Feeding frequency, and amount and type of food
TEST ORGANISMS
1. Scientific name and how determined 2. Age at test initiation 3. Life stage 4. Mean length and weight (where applicable) 5. Source 6. Diseases and treatment (where applicable) 7. Taxonomic key use for species identification
QUALITY ASSURANCE
1. Reference toxicant used routinely; source 2. Date and time of most recent reference toxicant test, test results, and current control chart 3. Dilution water used in reference toxicant test 4. Results (NOEC or, where applicable, LOEC, LC50, EC50, IC25 and/or IC50) 5. Physical and chemical methods used
RESULTS
1. Raw toxicity data in tabular form, including daily records of affected organisms in each concentration (including controls), and plots of toxicity data 2. Table of LC50s, NOECs, IC25, IC50, etc. 3. Statistical methods used to calculate endpoints 4. Summary table of physical and chemical data 5. Tabulate QA data

Figure 9-1. Summary of Minimum Laboratory Data Report Contents

SECTION 10
DATA REPORTING PROTOCOLS

All data collected as part of a Caltrans Stormwater Monitoring project are entered into the Caltrans Statewide Stormwater Database. To facilitate data management, analysis, and the comparison of results from Caltrans Districts throughout the State, a uniform system for data reporting is required for all Caltrans monitoring projects, including toxicity studies. Every fall, and periodically each spring, Caltrans distributes a Data Reporting Protocols document that describes the manner in which data should be entered into the Database. The most recent version of the Data Reporting Protocols (Caltrans, 2001), includes protocols for toxicity data, sediment data, and litter data in addition to water quality data. The data reporting protocols specific to toxicity results are presented in this section, along with general instructions for all stormwater data entered in the Caltrans Stormwater Database and the general organization of the database. These specific topics are covered in the following order:

- **General Instructions**
- **Organization of the Caltrans Statewide Stormwater Database**
- **List of Important Fields for the Toxicity Portion of the Database**
- **Overview of Toxicity Data Analysis**

GENERAL INSTRUCTIONS

Every monitoring site is assigned an ID number by Caltrans. At the beginning of each monitoring season, data reporters must contact the Caltrans database manager to receive the Site ID for each site to be sampled. Data reporters provide the name of the site, Caltrans District and constituents to be monitored. The database manager then supplies the appropriate Site IDs, or assigns them, if previously unmonitored sites are added to a project.

Standardized entries are provided for almost every field. These entries must be used exactly as presented in the Protocols (including for example specifications for spacing, hyphenation or capitalization), and attention must be given to the units specified for each constituent. If the standardized list does not contain an appropriate descriptor, the data reporter should contact the database manager prior to submitting any non-standardized entries.

The database manager will provide every data reporter with a data-reporting worksheet (Excel file format) in which all data must be submitted. All data fields should be included, even when they are left blank. Time series data (for example flow, precipitation, etc.) should be presented in a separate Excel worksheet, one for each site. For detailed instructions on how to include time series data, see Part II, Caltrans

Monitoring Protocols Guidance Manual: Water Quality (Caltrans, 2000a). For the reporting of dates and times, specific formats and references are dictated in the Protocols, as is a definition of a precipitation event. The purpose is to provide consistency in data reporting and calculation of summary statistics.

ORGANIZATION OF THE CALTRANS STATEWIDE STORMWATER DATABASE

The aquatic toxicity database is separated into four parts: Sample Description, Sampling Event Description, Site Descriptions, and Aquatic Toxicity Sample Descriptions. All four sections contain an Event ID and Monitoring Site ID field.

The Sample Description portion of the database allows for input of standard water quality information associated with the toxicity samples. These data can be instrumental in interpreting potential toxicity. The information included in this portion of the database describes the sample itself: when and how it was collected, what it was analyzed for, the method and lab used to perform the analysis, and the result of the analysis. This section also allows the data reporter to characterize the sample source, as well as the portion of a rain event that is represented by the sample.

The second part of the Database contains data that describes the precipitation event itself. This includes when the rain started and stopped, rainfall intensity, when runoff started and ended, the total amount of rainfall prior to, and during, the event, and antecedent dry days.

In the third section of the Database, the data reporter enters records that describe the site at which the sample was obtained. The fields span a range of categories from geographic information and boundaries, such as coordinates, hydrologic sub-area, land use, and size of the watershed, to political data like county, Caltrans and Regional Water Quality Control Board (RWQCB) district.

The Aquatic Toxicity Sample Description portion of the database allows the data reporter to submit information that describes the sample itself: when and how it was collected, what tests were performed, the method and lab used to perform the testing, and the test results. This section also allows the data reporter to characterize the sample source, as well as the portion of a rain event that is represented by the sample.

LIST OF IMPORTANT FIELDS WITHIN THE TOXICITY PORTION OF THE DATABASE

This section lists the data fields in the Database specific to toxicity test results. It is not meant to reproduce the Protocols, but simply to give the reader an idea of the type of information that is required from data reporters.

The Sample Description section for the toxicity portion of the database differs significantly from the Sample Description portion of the water quality database. Although some of the fields are the same (e.g. event identification, site identification, sample start and end time and date, collection method, etc.) several additional fields have been added. Database fields specific to toxicity test results include information such as test organism, test type, result type, observed statistically significant differences, whether or not TIEs were performed, and if TIEs successfully identified toxic constituents. The specific data fields included in the Sample Description section of the toxicity portion of the database are presented in the example spreadsheet included as Figure 10-1. Examples of data that may be entered into each of the data fields are also included in Figure 10-1.

The Sampling Event description section is identical to the water quality portion of the database which includes such fields as the Rain Start Time, Rain End Time, Rain Start Date, Rain End Date, Event Rain, Max Intensity, Total Flow Volume, Peak Flow and the Estimated % Capture, as well as who collected the sample.

The Site Description portion is also identical to the water quality database fields, which include the Caltrans District, County, RWQCB, Latitude, Longitude, Land Use, Catchment Area, Impervious Fraction, Post Mile, Receiving Water Type, Time Series, and Site Description.

OVERVIEW OF TOXICITY DATA ANALYSIS

Toxicity data can be expressed and reported in several ways. This section provides an overview of acute and chronic toxicity data analysis and the ways in which toxicity data are reported. The possible result types for each toxicity test method are presented in Table 10-1.

Acute Toxicity Data Analysis

Analysis of acute toxicity data will depend on the type of test that is conducted (i.e. screening level or dilution series, see ***Section 4***). For screening level tests, results are analyzed by hypothesis testing. Hypothesis testing in this case is used to determine if survival in the sample is significantly different from control survival. For tests with a dilution series, results may be analyzed by hypothesis testing or used to determine a lethal concentration$_{50}$ (LC_{50}) or other point estimate (e.g. LC_{10}, LC_{25}). An LC_{50} refers to an estimate of the concentration that is lethal to 50 percent of the test organisms. U.S. EPA guidance describes four possible methods for estimating the LC_{50} including the Graphical Method, Spearman-Karber Method, the Trimmed Spearman-Karber Method, and the Probit Method. The requirements, description of calculations involved or computer program used, and an example of the calculations for each of these methods are described in U.S. EPA (1993) guidance. Hypothesis testing for dilution tests is conducted to determine the No Observed Effect Concentration (NOEC). An NOEC represents the highest measured concentration of a sample or toxicant that causes no statistically significant adverse effect on test organisms.

Results may also be reported in toxic units (TU). The toxic unit for an individual acute toxicity test (TUa) or chronic toxicity test (TU_c) is calculated by taking the reciprocal of the sample dilution that causes the effect (i.e. the LC_{50}, LC_{25}, or NOEC) by the end of the acute exposure period. An example TU calculation is illustrated below:

$$TU = \frac{\text{highest sample concentration tested (100\%)}}{LC_{50},\ LC_{25},\ \text{or NOEC}}$$

Chronic Toxicity Data Analysis

Chronic toxicity tests measure lethality as well as other endpoints including reproduction for *Ceriodaphnia dubia*, growth for *Pimephales promelas*, and cell growth for *Raphidocelis subcapitata*. Statistical analysis for each of these tests is described below.

Ceriodaphnia dubia

Point estimation techniques are used to determine the EC_{25}, EC_{50} (EC values represent the estimated concentration that effects survival of a certain percentage (25, 50, etc.) of test organisms), and LOEC and NOEC values for survival and reproduction are obtained using a hypothesis test approach such as Fisher's Exact Test, Dunnett's Procedure, or Steel's Many-one Rank Test. Separate analyses are performed for the estimation of the LOEC and NOEC endpoints and for the estimation of EC_{25}, and EC_{50}.

The response used in the statistical analysis for survival is the number of animals surviving at each test concentration. Concentrations at which there is no survival in any of the test chambers are excluded from the statistical analysis of the NOEC and LOEC for reproduction, but included in the estimations of the EC_{25} and EC_{50}. The response used in the analysis for reproduction is the number of young (neonates) produced per adult female, which is determined by taking the total number of young produced until either the time of death of the adult or the end of the experiment, whichever comes first. An animal that dies before producing young, if it has not been identified as a male, would be included in the analysis with zero entered as the number of young produced. The subsequent calculation of the mean number of live young produced per adult female for each toxicant concentration provides a combined measure of the toxicant's effect on both mortality and reproduction.

Table 10-1. Summary of Result Types for Acute and Chronic Toxicity Tests

Test Method	Endpoint(s) Tested	Result Types (units)	
Test Type		**Screening Level**	**Dilution Series**
Acute *Ceriodaphnia dubia, Pimephales Promelas,* and *Oncorynchus mykiss*	Mortality	• Mean mortality (%) • Statistically significant difference from control (yes/no) • Standard error (%)	• Mean mortality (%) • Standard error (%) • LC_{50} (% dilution) • NOEC/LOEC (% dilution) • Acute Toxic Units (TUa)
Chronic *Ceriodaphnia dubia, Pimephales promelas*, and *Oncorynchus mykiss*	Mortality	• Mean mortality (%) • Statistically significant difference from control (yes/no) • Standard error (%)	• Mean mortality (%) • Standard error (%) • LC_{50} (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Ceriodaphnia dubia*	Reproduction	• Mean reproduction (neonates/adult) • Statistically significant difference from control (yes/no) • Standard error (neonates/adult)	• Mean reproduction (neonates/adult) • Standard error (neonates/adult) • $EC_{50,}$ EC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Pimephales promelas*	Growth	• Mean growth (mg/individual) • Statistically significant difference from control (yes/no) • Standard error (mg/individual)	• Mean growth (mg/individual) • Standard error (mg/individual) • $IC_{50,}$ IC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Selenastrum capricornutum*	Growth (cell number)	• Mean growth (cells/mL) • Statistically significant difference from control (yes/no) • Standard error (cells/mL)	• Mean growth (cells/mL) • Standard error (cells/mL) • $IC_{50,}$ IC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)

Pimephales promelas

Point estimation techniques are used to determine the LC_{50}, IC_{25}, and IC_{50} values . Lowest observed effect concentration (LOEC) and NOEC values for survival and growth are obtained using a hypothesis testing approach such as Dunnett's Procedure or Steel's Many-one Rank Test. Separate analyses are performed for the estimation of the LOEC and NOEC endpoints and for the estimation of LC_{50}, IC_{25}, and IC_{50}.

The response used in the statistical analysis of survival is the number of animals surviving at each test concentration. Concentrations at which there is no survival in any of the test chambers are excluded from statistical analysis of the NOEC and LOEC, but included in the estimation of IC and LC endpoints. The response used in the statistical analysis of growth data is mean dry weight per replicate. An IC estimate can be calculated for the growth data via point estimation techniques. Hypothesis testing can be used to obtain the NOEC for growth. Concentrations above the NOEC for survival are excluded from the hypothesis test for growth effects.

Selenastrum capricornutum

The IC_{25} and IC_{50} are calculated using the point estimation techniques, and LOEC and NOEC values for growth are obtained using a hypothesis testing approach such as Dunnett's Procedure or Steel's Many-one Rank Test. Separate analyses are performed for the estimation of the LOEC and NOEC endpoints and for the estimation of IC values.

The response used in the statistical analysis for this test is the number of cells per milliliter per replicate.

Sample ID	Treatment	Test Type	Sample Date	Test start date	Test end date	Test organism	Parameter	Result Type	Units	Num Qual	Result	Statsig	Test Day	TIE performed	Suspect Toxicant(s)	TIE Phase Completed
CT-1	none	acute	4/30/02	5/1/02	5/3/02	*C.dubia*	mortality		%		30		2	no	no	I
CT-1	100 ppb PBO	acute	4/30/02	5/1/02	5/3/02	*C.dubia*	mortality	signif.	yes/no		no	no	2			
CT-10	none	chronic	8/6/02	8/7/02	8/14/02	*P. promelas*	growth	NOEC	%	<	6	yes	7	yes	copper	III
CT-10	none	chronic	8/6/02	8/7/02	8/14/02	*P. promelas*	growth	signif.	yes/no			yes				

Figure 10-1. Example of Toxicity Data Fields

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APPENDIX A
TOXICITY IDENTIFICATION EVALUATIONS (TIEs)

This appendix provides specific information on TIE manipulations. Text was extracted , with a few modifications, from the technical report developed by Kinnetic Laboratories, Inc. for Caltrans (Caltrans, 1999b). Information presented in this write-up is based on an extensive review of the available runoff toxicity data (Caltrans, 1999c). The purpose of this appendix is to provide background information on TIEs and to outline, as an example **ONLY,** the TIE methods that may be appropriate for stormwater samples. This information is included to simply illustrate that TIEs for stormwater samples is a complex process and adjustments can be made based on the expertise and best professional judgement of the toxicity testing personnel. It is not the intent of this appendix to lay out the specific methods that should be followed for TIEs conducted for Caltrans toxicity studies.

TIEs

TIEs seek to identify toxiciants in a sample via a series of manipulations and associated bioassays which target a systematically narrowing list of suspect toxicants. Each manipulation is designed to identify a particular toxicant group by altering it or rendering it biologically unavailable. A series of EPA publications developed since 1988 by the EPA Environmental Research Laboratory - Duluth, MN form the basis for the TIE methodologies (U.S. EPA 1991, 1993b, 1993c). Three Phases comprise a full TIE:

Acute (lethality) or chronic tests can be used during the TIE characterizations. Final analysis of results compare endpoints (e.g. 96-hour LC_{50}s) from manipulated samples to those of an unaltered effluent baseline. Freshwater bioassay test species include the water flea *Ceriodaphnia dubia*, fathead minnow (*Pimephales promelas*) or alga (*Selenastrum capricornutum*).

Phase I (the Characterization phase) is aimed at developing information on the general chemical class of a contaminant (e.g. metals, non-polar organics, volatile compounds or pH-sensitive toxiciants) causing the toxicity (U.S. EPA, 1991). In this Phase, a toxic effluent is subjected to a series of chemical manipulations including pH adjustment, aeration, filtration, C_{18} (SPE) column extraction, chelation with EDTA, reduction of oxidants and evaluation of pH-dependent toxicity. Following each of the chemical manipulations, bioassays are performed to evaluate whether the toxicity has been reduced or eliminated.

The original EPA Phase I TIE procedures were designed for the characterization of municipal and industrial effluents (POTWs). However, since stormwater runoff is not likely to contain significant amounts of either chlorine or ammonia (expected in POTWs), and since prior TIEs of regional stormwaters have shown that evidence for a volatile toxicant has always correlated with evidence of a non-polar organic toxicant, we have

developed an abbreviated subset of the EPA procedures for stormwater samples. The graduated pH test, the oxidant-reduction test and the pH-adjustment test (to acid and alkaline extremes within each manipulation) have been eliminated from the Phase I TIE procedures. The toxicant groups targeted therefore are volatile or oxidizable compounds, particulate-bound toxins, cationic metals, non-polar organics, and organophosphates:

- Aeration of the sample sparges volatiles and will also oxidize some compounds.

- Filtration identifies the extent to which sample toxicity is affected by particulates, particulate-bound toxicants or compounds in suspension.

- C_{18} SPE uses a sorbent column to evaluate the contribution to sample toxicity of non-polar organic compounds and certain metals or metal chelates. Non-polar compounds are trapped in the column through solubility and polarity interactions with the C_{18}.

- EDTA reduces sample toxicity by chelation of certain cationic metals. For Ceriodaphnia EDTA chelation has been shown to effectively remove or reduce the toxicity of Cd^{++}, Cu^{++}, Pb^{++}, Mn^{++}, Ni^{++}, and Zn^{++}. If cationic metals are present in the sample, toxicity should be diminished by small additions of EDTA but may be increased by larger additions, due to toxicity of the EDTA itself.

- Piperonyl Butoxide (PBO) blocks the action of metabolically-activated organophosphate compounds (OP pesticides). The addition of PBO therefore reduces toxicity of samples containing Ops. PBO however, also enhances the toxicity of pyrethroid insecticides.

At the Phase I level of testing, relatively small sample sizes and limited replications make statistical inferences difficult. Often 95% confidence limits on LC_{50} values are not calculable, and thus the statistical significance of substantial changes in post-manipulation toxicity may not be possible to establish. This is especially true in cases of samples with low to moderate initial 96h toxicity (LC_{50} >50%). Therefore, considerable latitde must be given when interpreting the results of Phase I TIEs.

Phase II (the Identification Phase) is designed to identify specific toxicants within the toxicant class or classes implicated during the Phase I testing (U.S. EPA, 1993b). The complexity of Phase II is heavily dependent upon which toxicant classes have been implicated. For example, if Phase I has indicated a metal toxicant, then Phase II less difficult. The test effluent is analyzed for metals content, and the analytical results compared with available metals toxicity criteria data for the test species used. If non-polar organic compounds are suspected, Phase II is considerably more complex. The effluent is subjected to a series of SPE and HPLC fractionations, concentration and toxicity testing, combined with GC/MS or other analysis until particular toxicants are identified and quantified. Each step in the series is designed to narrow the possible

suspects; then, based on the quantitative analytical identifications, the toxicity results are compared with the known dose-responses of the short-list of suspects.

Phase II Non-Polar Organics

Our procedures follow those of EPA (U.S. EPA, 1993b), but also draw heavily on Bailey, et al. (1996). The approach is one of successive steps of fractionation, methanol elution and concentration, each followed by toxicity tests and chemical analyses until suspect toxicants are identified and compared with toxicity values. The following paragraphs summarize the methods employed during Phase II non-polar organic investigations.

- HPLC Fractionation. MEOH fractions which demonstrate toxicity, and their corresponding MEOH blank fractions are further concentrated, then fractionated using High Performance Liquid Chromatography (HPLC). For each sample, this produced a total of 30 concentrated (615x-666x) subfractions which are tested for toxicity.

- pH adjustments. Whole sample is adjusted to pH 3 and pH 11, using HCl and NaOH. Adjusted sample is held for 6 h, then re-adjusted back t o initial pH_i. Toxicity testing is then conducted on the two adjusted/re-adjusted samples.

- Analytical Chemistry. The original (unmanipulated) samples are extracted and analyzed by GC or GC/MS for suspect organic toxicants. The HPLC fractions are also analyzed by these methods.

Phase II Metals

The EPA Phase II approach for the identification of suspect metals toxicants include a suite of procedures based initially on analysis for metals in the sample, and comparison of the metals analysis data with the sample toxicity test results and with published metals toxicity information. Comparisons are made with published toxicity-based water quality criteria (SFBRWQCB, 1991 and U.S. EPA, 1993a), and to species-specific metals toxicities [(e.g. Ceriodaphnia (Schubauer-Berigan et al. 1993)]. Ion-exchange resins have also been used to provide supporting evidence for identifying the cause of toxicity in effluents (U.S. EPA, 1993b).

Phase II Volatiles, Oxidizables, Surfactants

To test for Contributions of volatile, oxidizable or surfactant compounds to toxicity, aliquots of original sample are aerated with air and with nitrogen, then toxicity tested. Aliquots are also created from rinsate of the aeration container walls, which may harbor surfactants from the sample.

Phase III (the Confirmation Phase) serves to verify the conclusions reached in Phase II by repeating the critical Phase II work with elevated levels of QA/QC during the testing process (U.S. EPA, 1993c). Phase III can, in some ways, be a formality, since some confirmation steps are inherent in the Phase II treatments. There may be no real "boundary" between Phase II and Phase III. Therefore, the Toxicity Confirmation Procedures (Phase III) manual is written as a series of "approaches" to the problem of toxicity confirmation; e.g. the correlation approach, the symptom approach, the species sensitivity approach, the spiking approach, and the mass balance approach. The final choice of our approach(es) to the confirmation task depends, in many ways, on the results of Phase II.

APPENDIX B

OCTOBER 2001

Caltrans Storm Water Management Program

2001-2002 Toxicity Data-Reporting Protocols

CTSW-TM-01-060

Prepared for:
California Department of Transportation
Environmental Program

GENERAL INSTRUCTIONS

(10/15/01)

Please read and be familiar with these general instructions. Toxicity and associated water quality data can only be accurately and expediently incorporated into the Caltrans Statewide Storm Water Database if these instructions are carefully followed. Adhering to these instructions will facilitate the processing of data and eliminate the need for time-consuming "record-by-record" data validation at year's end.

- **Get Monitoring Site IDs from Caltrans at the beginning of each monitoring season.**

  Caltrans will assign the "Monitoring Site ID". All Caltrans data reporters are required to contact the Caltrans database manager at the beginning of each sampling season to receive Monitoring Site IDs for each new site they will sample. Note that BMP sites having multiple points of collections (e.g., inlet, within, outlet) will be assigned a unique Monitoring Site ID for each point of collection. Data reporters shall provide the database manager with the names of each site, the Caltrans district in which each site resides, and the constituents that will be monitored at each site.

- **Enter data appropriately.**

  To facilitate the accurate entry of water quality data into Excel worksheets by Caltrans data reporters, and the subsequent transfer of this data into the Caltrans Statewide Storm Water Database, each data field described in this data-reporting protocol document possesses the following attributes: Field Name, Field Type, Field Size, Example, Definition, Notes (where applicable), Standard List (where applicable) and Alternate Value (where applicable). These eight data field attributes are defined below.

Field Name	Name of data field as it appears in the Excel data-reporting worksheet.
Field Type	Specifies the type of data, text or numeric, that can be stored in a field. Note: text fields can store both text and numbers.
Field Size	Specifies the number of characters that can be stored in a text field, or the number of decimal places required of a number entered into a numeric field.
Example	Provides an example of the data type and possible value that is appropriate for a specific data field.
Definition	Provides a definition of the data type and possible value that is appropriate for a specific data field.
Notes	Provides further information that may be helpful in appropriately and accurately populating a specific data field.
Standard List	Provides a list of standard values appropriate for populating a specific data field.
Alternate Value	Specifies if the use of "N/A" (not applicable) or "None" is an appropriate value for a specific data field.

Additionally, the following rules should be abided by when entering toxicity and associated water quality data into the Excel data-reporting worksheets provided by Caltrans.

- Select and enter text values only from the "Standard List" of values provided for each data field. Standardized values must be entered exactly as specified. Standard lists are included with the description of each data field where appropriate. If a standardized value provided for a particular data field does not accurately describe the data to be entered into that field, please contact the Caltrans database manager to discuss the creation of an additional value for a particular data field.

- **Use "Constituent"** names exactly as they appear in the constituent list, and report data in the units specified. Please refer to Section 4 at the end of these protocols for the list of "Standard Constituent Names".

- Enter numeric data values according to the definition provided for the particular numeric data field. Text must not be entered into numeric data fields. Text-based comments regarding a numeric value should be placed in the "Notes" data field.

- "Event ID" follows the format **YYYY-NN** ("Year monitoring season begins-Number of event at site beginning with 01"). Event IDs for the 2001-2002 Caltrans monitoring season would all begin with "2001". The first event monitored at any site shall be assigned an Event ID of "2001-01". Additional events at a particular site shall be numbered consecutively beginning with "2001-02".

- Use the correct system for date entries. Dates must be entered using the Excel "1900 Date System" typical for Windows (the "1900 Date System" is the default date system used by Microsoft Excel installed on Windows machines). The "Macintosh 1904 Date System" should be switched off in Excel preferences (located in the following nested menus: Tools >> Options >> Calculation).

- Values contained in data fields that link records from one Excel worksheet (or Access table) to another, such as Monitoring Site ID and Event ID, must match exactly for the correct relationship to exist between records once the data is imported into the Caltrans Statewide Storm Water Database (Version 2.0 built using Microsoft Access 2000). No match can be made between a Monitoring Site ID of "7 -21" (note an extra space between the 7 and the hyphen) found in the Site Descriptions worksheet and a Monitoring Site ID of "7-21" found in the Sample Descriptions worksheet. In general, wherever data field entries are hyphenated, do not include spaces.

- If any cell in a data field is left blank* (i.e., the data cell is null), a brief note explaining why the data cell was left blank must accompany the particular record. The brief note shall be placed in the "Notes" data field associated with the particular record containing one or more blank data cells.

  * Exceptions – The following data fields can contain null values without an associated note entered in the "Notes" data field of the relevant worksheet or data table:

Worksheet	Data Field
Sample Descriptions	Overall Qualifier
Aquatic Toxicity Sample Descriptions	Statistical Significance (with any result type except statistically significant difference).

- The use of "N/A" as a value entered into a text data field is acceptable for some fields where the data field definition does not apply to the particular data record being entered. For example, the data field "Control Site" (found in the Site Descriptions worksheet) applies only to Caltrans BMP sites for which a control site exists. This data field is "not applicable" to a non-BMP monitoring site with a "Point of Collection" equal to "Discharge". Data fields where the use of "N/A" is acceptable have this data value option specified in the Alternate Value attribute. "N/A" cannot be entered into a numeric data field. (***Additionally, the use of the value "Other" is no longer acceptable for any data field).***

- **Make sure that all required data records for a particular sampling event exist in the Sample Descriptions, Sampling Event Descriptions and Site Descriptions worksheets according to the following rules to maintain referential integrity among all related records:**

  - A Monitoring Site ID value (e.g., 7-21) contained in the Sample Descriptions worksheet must be represented by a single record for the same Monitoring Site ID in the Site Descriptions worksheet.

  - A Monitoring Site ID value (e.g., 7-21) contained in the Site Descriptions worksheet must be represented by at least one record in the Sample Descriptions worksheet.

  - A unique combination of Monitoring Site ID (e.g., 7-21) and Event ID (e.g., 2001-01) contained in the Sample Descriptions worksheet must be represented by a single record for the same unique Monitoring Site ID/Event ID pair in the Sampling Event Descriptions worksheet. Do not forget to create an Event Descriptions record for "non-storm" monitoring events (such as dry weather sampling, groundwater sampling, etc.) even though these events may not include the collection of precipitation and runoff data (Event ID, Site ID, Event Type, Sample Collector and Organization can be populated for these non-storm event records).

  - A unique combination of Monitoring Site ID (e.g., 7-21) and Event ID (e.g., 2001-01) found in the Sample Event Descriptions worksheet must be represented by at least one record in the Sample Descriptions worksheet having the same Monitoring Site ID/Event ID pair.

- **Do not report analytical results for blanks, duplicates, or reference toxicants. Report only values from environmental samples that have been QA/QC'ed.**

- **Use only Excel worksheets provided by Caltrans for the entry of water quality data.**

  All "Electronic Data Deliverables" (EDD) must be submitted in the format of the data-reporting worksheets provided by Caltrans. All data field headings should remain as they were originally entered into the worksheets at the time of their distribution to data reporters. Confirm that all data entered in the worksheets correspond to the appropriate data field headings.

- **Label all elements ofeach Electronic Data Deliverable (EDD) clearly**

  - The Excel workbook file containing worksheets storing Sample Descriptions, Sampling Event Descriptions, and Site Descriptions data should be labeled with the name of the data reporter (consultant or university), the title of the Caltrans activity under which the data was collected, and the monitoring season (e.g., 2001-2002 or 01-02) during which the data was collected.

    File naming convention for water quality data:

Water quality data file	CDM Dice Study_2001-2002
	UCLA First Flush Study_01-02

  - The submittal of supporting data and documents, such as time series data, maps, and photographs, should be labeled with the Caltrans Monitoring Site ID and monitoring season to which they correspond. Times series data collected during storm events at a monitoring site can be submitted as multiple worksheets (one worksheet for each storm event) in a single workbook that represents all times series data collected at that site during an entire monitoring season.

    File naming conventions for supporting data:

Time series file	Site 7-21 Time Series_2001-2002.xls
Map file	Site 7-21 Map_2001-2002.pdf
Photograph file	Site 7-21 Photo_2001-2002.jpeg

  - When submitting an EDD to the Caltrans database manager, please compile all files (toxicity and associated water quality data, including sample, event and site descriptions, time series data, maps and photographs) onto one or more recordable compact discs (CD-R) labeled with the data reporter's name and date of data submittal. Mail CD(s) to the database manager at the address provided below.

- **Please notify Caltrans of any errors or inconsistencies found in this document, or any changes you might recommend.**

Definition of a Precipitation Event

For the purposes of these protocols, a precipitation event shall begin with six consecutive hours during which a sum total of at least 2.54 mm (0.1 inches) of rain falls, and end with six consecutive hours in each of which no rainfall greater than 0.254 mm (0.01 inches) of rain is recorded. The precipitation event so identified shall be truncated so that it both begins and ends in hours with rainfallequal to or greater than 0.254 mm (0.01 inches).

Summary Toxicity Information

Table 1 below presents a summary of associated toxicity data. This table should serve as a reference when inputing data into the aquatic toxicity sample description portion of the database.

GENERAL INSTRUCTIONS (CONTINUED)

Test Method	**Endpoint(s) Tested**	**Result Types (units)**	
Test Type		**Screening Level**	**Dilution Series**
Acute *Ceriodaphnia dubia*, *Pimephales Promelas*, and *Oncorynchus mykiss*	Mortality	• Mean mortality (%) • Statistically significant difference from control (yes/no) • Standard error (%)	• Mean mortality (%) • Standard error (%) • LC_{50} (% dilution) • NOEC/LOEC (% dilution) • Acute Toxic Units (TUa)
Chronic *Ceriodaphnia dubia*, *Pimephales promelas*, and *Oncorynchus mykiss*	Mortality	• Mean mortality (%) • Statistically significant difference from control (yes/no) • Standard error (%)	• Mean mortality (%) • Standard error (%) • LC_{50} (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Ceriodaphnia dubia*	Reproduction	• Mean reproduction (neonates/adult) • Statistically significant difference from control (yes/no) • Standard error (neonates/adult)	• Mean reproduction (neonates/adult) • Standard error (neonates/adult) • $EC_{50,}$ EC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Pimephales promelas*	Growth	• Mean growth (mg/individual) • Statistically significant difference from control (yes/no) • Standard error (mg/individual)	• Mean growth (mg/individual) • Standard error (mg/individual) • $IC_{50,}$ IC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)
Chronic *Selenastrum capricornutum*	Growth (cell number)	• Mean growth (cells/mL) • Statistically significant difference from control (yes/no) • Standard error (cells/mL)	• Mean growth (cells/mL) • Standard error (cells/mL) • $IC_{50,}$ IC_{25} etc. (% dilution) • NOEC/LOEC (% dilution) • Chronic Toxic units (TUc)

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1. WATER QUALITY SAMPLE DESCRIPTIONS

1.1. Monitoring Site ID

Field Type: Text
Field Size: 8 characters
Example: 7-21
Definition: A unique identification number assigned to the monitoring site by Caltrans.
Notes: The first part of the Monitoring Site ID corresponds to the Caltrans district in which the site is located.

1.2. Event ID

Field Type: Text
Field Size: 8 characters
Example: 2001-01
Definition: A unique, site-specific identification number assigned to a sampling event.
Notes: Event ID follows the format YYYY-NN ("Year monitoring season begins-Number of event at site beginning with 01"). The first event at any site would be assigned an Event ID of "Year-01". Additional events at a particular site shall be numbered consecutively beginning with "Year-02". Even if a site isn't monitored until the latter half of the monitoring season (e.g., Site X is monitored during Feb. – Apr. 2002 – the latter half of the 2001-2002 monitoring season), its Event IDs include the year in which the general Caltrans monitoring season began (e.g., samples collected during the first event at Site X in Feb. 2002 are assigned Event IDs of "2001-01").

1.3. Sample Start Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which composite sampling begins or grab sample is collected. In regard to composite samples, specifically the date on which the sampler was activated <u>and</u> the flow meter began totalizing flow.
Notes: Formatted as mm/dd/yyyy.

1.4. Sample Start Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which composite sampling begins or grab sample is collected. In regard to composite sampling, specifically the time at which the sampler was activated <u>and</u> the flow meter began totalizing flow.
Notes: Formatted for 24-hour clock (hh:mm).

1.5. Sample End Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which composite sampling ends. Specifically, the date on which the sampler was de-activated and/or the flow meter stopped totalizing flow.
Notes: Formatted as mm/dd/yyyy.

1.6. Sample End Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which composite sampling ends. Specifically, the time at which the sampler was de-activated and/or the flow meter stopped totalizing flow.
Notes: Formatted for 24-hour clock (hh:mm).

1.7. Sample Source

Field Type: Text
Field Size: 12 characters
Example: Storm
Definition: Identifies the source of the water quality sample analyzed.
Notes: Standardized Entry. Enter value from standard list or contact database manager.

Standard List

- Storm - Sample from storm water(rainfall) runoff
- Rain - Sample from rainwater collected directly from the atmosphere
- Receiving - Sample from receiving water
- Vadose - Sample from vadose zone
- Sediment - Sample from deposited sediment
- Base - Sample from dry-weather base flow
- Groundwater - Sample from groundwater
- Non-storm - Sample from non-storm water runoff
- BMP media - Sample from material used to construct BMP device

1.8. Event Representation

Field Type: Text
Field Size: 12 characters
Example: FF
Definition: Describes the part of the event represented by the sample.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- FF - Event first flush
- Peak - Event peak flow
- Whole - Whole storm
- Discrete - One or more discrete samples collected during an event that cannot be described as representing the first flush, peak or whole storm portion of the event. This value will typically, although not exclusively, be used for a "Non-storm" Sample Source.
- X-hr Comp - Used to describe a planned composite sampling event lasting X hours.

1.9. Sample Type

Field Type: Text
Field Size: 2 characters
Example: C
Definition: Describes the way in which sample was collected.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- C - Composite Sample: A sample made up of multiple sub-samples (aliquots) collected over some spatial or temporal scale.
- G - Grab Sample: A single sample that represents conditions at a distinct point in time.

1.10. Sample Matrix

Field Type: Text
Field Size: 10 characters
Example: Water
Definition: Matrix from which sample was analyzed.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- Water

Note: Other sample matrices, such as sediment, sludge, soil, solid, etc. are now included in the 2001-2002 Sediment Data-Reporting Protocols document.

1.11. Constituent Type

Field Type: Text
Field Size: 4
Example: CON
Definition: Describes the type or class of constituent analyzed.
Notes: Standardized Entry. Enter value from standard list or contact database manager.

Standard List

- CON - Conventionals
- MIN - Minerals
- N - Nutrients

1.12. CAS Number

Field Type: Text
Field Size: 15 characters
Example: 7440-50-8
Definition: CAS Number (Chemical Abstracts Service Registry Number) or Caltrans Constituent ID number for constituent.

Standard List

- Alkalinity as CaCO3 CT-ALKCACO3
- DO CT-DO
- EC (µmhos/cm) CT-EC
- Hardness as CaCO3 CT-HARD
- pH (pH units) CT-PH
- Temperature (^{o}C) CT-TEMP
- Chlorine Residual CT-CLRES
- NH3-N CT-NH3N

1.13. Constituent

Field Type: Text
Field Size: 40 characters
Example: Cu
Definition: Name of constituent as found in the Caltrans Statewide Storm Water Database.
Notes: Standardized Entry. Enter value from standard list or contact database manager.

Standard List

- Alkalinity
- DO
- EC
- Hardness
- pH
- Temperature
- Chlorine Residual
- NH3-N

1.14. Fraction

Field Type: Text
Field Size: 6 characters
Example: N/A
Definition: Describes fraction of constituent analyzed.
Notes: Standardized Entry. Dissolved or total fractions are not applicable to the analytical constituents associated with toxicity samples (as listed above in 1.13). However, THIS DATA FIELD CAN NO LONGER BE LEFT BLANK/NULL. Therefore, these constituents should receive a value of "N/A" (not applicable) to describe their fraction analyzed.

1.15. Numerical Qualifier

Field Type: Text
Field Size: 1 character
Example: <
Definition: An indication of what the Reported Value for an analyzed sample represents (i.e., a minimum, maximum or "exact" value).
Notes: Standardized Entry. Enter value from standard list or contact database manager. Please refer to Overall Qualifier (see data field 1.17. below) for additional guidance on the appropriate qualification of water quality results.

Standard List

- < - Actual value of analyzed sample is less than reported value. This symbol must be used for all "non-detect" samples.
- = - Value of analyzed sample is as reported.
- > - Actual value of analyzed sample is greater than reported value.

1.16. Reported Value

Field Type: Numeric
Field Size: n/a
Example: 0.25
Definition: Reported laboratory result of analyzed sample.
Notes: Reported Value must equal Reporting Limit if associated Numerical Qualifier is "<".

1.17. Overall Qualifier

Field Type: Text
Field Size: 4 characters
Example: U
Definition: An overall data validation qualifier based on lab results and associated quality assurance/quality control (QAQC) analyses.
Notes: Standardized Entry. Enter value from standard list or contact database manager. The qualifiers and definitions presented below represent a compilation of data qualifiers used by specific EPA programs as noted in: (1) *USEPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review* (EPA, 1994); and (2) *USEPA Contract Laboratory Program National Functional Guidelines for Organic Data Review* (EPA, 1999).

Standard List

- U - The material was analyzed for, but was not detected above the level of the associated value (the associated value represents a reporting limit that may or may not be elevated due to blank contamination).
- J - The associated value is approximate (i.e., an estimated quantity).
- N - The analysis indicates the presence of an analyte for which there is presumptive evidence to make a "tentative identification".
- NJ - The analysis indicates the presence of an analyte that has been "tentatively identified" and the associated numerical value represents its approximate concentration.
- UJ - The material was analyzed for, but was not detected. The associated value (i.e., reporting limit) is an estimate and may be inaccurate or imprecise.
- R - The sample result is rejected due to serious deficiencies in the ability to analyze the sample and meet quality control criteria. The presence or absence of the analyte cannot be verified.

1.18. Units

Field Type: Text
Field Size: 12 characters
Example: mg/L
Definition: Describes the unit of measure associated with the Reported Value.
Notes: Standardized Entry. Enter value from standard list or contact database manager. Report sample results in the appropriate unit associated with a particular constituent as specified in the standardized list. Use capital "L" for liter abbreviation. Confirm that values for Reported Value, Method Detection Limit, and Reporting Limit are all reported in the same units for individual sample records.

Standard List

- Alkalinity mg/L
- DO mg/L
- EC µmhos/cm
- Hardness mg/L
- pH pH units
- Temperature ^{o}C
- Chlorine Residual mg/L
- NH3-N mg/L

1.19. Preparation Method

Field Type: Text
Field Size: 40 characters
Example: Separatory Funnel
Definition: Describes the preparation a sample underwent prior to analysis. All samples receiving no form of preparation prior to analysis should have this data field value reported as "None".
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- Hot Plate
- Microwave
- Purge and Trap
- Methanol Extraction
- Continuous
- Separatory Funnel
- Soxhlet
- Ultrasonic Extraction
- Solid Phase Extraction - for water samples
- Accelerated Solvent Extraction

Alternate Value: - None (see Definition above).

1.20. Method Reference

Field Type: Text
Field Size: 10 characters
Example: EPA
Definition: Provides the reference for the analytical method used to evaluate the sample.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- EPA - US Environmental Protection Agency.
- SM - Standard Methods.
- Field - Constituent evaluated in the field. Note: no Method Numbers are associated with field-measured constituents.
- Caltrans - Caltrans-defined analytical method.

1.21. Method Number

Field Type: Text
Field Size: 10 characters
Example: 625
Definition: Reference number for the analytical method used to evaluate the sample.
Notes: By convention, method numbers that contain a text suffix do not include a space between the number and the text suffix (e.g., 6010A).

1.22. Method Detection Limit

Field Type: Numeric
Field Size: n/a
Example: 0.2
Definition: Method Detection Limit (MDL) – The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from the analysis of a sample in a given matrix containing the analyte.
Notes: Concentrations reported by a laboratory that are between the MDL and Reporting Limit (see data field 1.23. below) are usually flagged by the laboratory as estimated "J", indicating that the constituent is present but its concentration cannot be accurately quantified (see data field 1.17, Overall Qualifier, for further information on data validation qualifiers).

1.23. Reporting Limit

Field Type: Numeric
Field Size: n/a
Example: 1.0
Definition: Reporting Limit (RL) as reported by laboratory. This value is the lowest concentration of a constituent that can be reliably quantified within specified limits of precision and accuracy during routine laboratory operating conditions. Reporting Limit is equivalent to Practical Quantitative Limit (PQL) and Reported Detection Limit (RDL).
Notes: The RL may be elevated above the Caltrans "target" RL due to matrix interference.

1.24. Collection Method

Field Type: Text
Field Size: 6 characters
Example: Auto
Definition: Describes the way in which a sample was collected.
Notes: Standardized Entry. Select value from standard list or contact database manager.
<u>Standard List</u>

- Auto - Sample aliquot collected by automated sampling device.
- Manual - Sample aliquot collected manually.

1.25. Field Sample ID

Field Type: Text
Field Size: 35 characters
Example: 134-0010-281500A
Definition: Field identification number assigned to sample/sample vessel.
Notes: This data field can contain any combination of text and numeric characters.

1.26. Lab Name

Field Type: Text
Field Size: 25 characters
Example: APPL
Definition: Name of laboratory that analyzed sample.
Notes: Standardized Entry. Select value from standard list. The list of analytical laboratories used by the Caltrans Storm Water Management Program will likely change over time, and therefore the Standard List below might not include the name of every laboratory used in any given monitoring year.

Standard List

- APPL - Agriculture & Priority Pollutants Labs
- Aquascience
- Aquatic Bioassay and Consulting (ABC) Laboratory
- Associated Labs
- BSK
- CEL - Calscience Environmental Labs
- Del Mar Analytic
- GeoAnalytical Labs
- North Coast Labs
- Pat-Chem
- Quanterra
- Silliker
- Soil Control Lab
- ToxScan
- UCDATL -UC Davis Aquatic Toxicity Lab

1.27. Lab Sample ID

Field Type: Text
Field Size: 25 characters
Example: 120085-400A
Definition: Laboratory identification number assigned to sample by analytical laboratory.
Notes: This data field can contain any combination of text and numeric characters.

1.28. Contract Number

Field Type: Text
Field Size: 12 characters
Example: 43A005
Definition: Caltrans contract number under which sample was collected.
Notes: This data field can contain any combination of text and numeric characters.

1.29. Task Order Number

Field Type: Text
Field Size: 6 characters
Example: 11
Definition: Caltrans task order number under which sample was collected.

Notes: This data field can contain any combination of text and numeric characters.

1.30. Sample Notes

Field Type: Text
Field Size: 255 characters
Example: Sample End Time missing due to malfunctioning automated sampler.
Definition: This data field contains any notes or remarks about the sample or various data fields used to describe the sample.
Notes: Data reporter should provide a note for any data field left blank (with the exception of Overall Qualifier). It is not appropriate to provide a note in a particular cell of a data field other than Sample Notes. Considering the example above, it is not appropriate to place the note, "Sample End Time missing due to malfunctioning automated sampler", in the Sample End Time data field. The note must be entered into the Sample Notes data field.

2. WATER QUALITY SAMPLING EVENT DESCRIPTIONS

2.1. Event ID

Field Type: Text
Field Size: 8 characters
Example: 2001-01
Definition: A unique, site-specific identification number assigned to a sampling event.
Notes: Event ID follows the format "Year monitoring season begins-Number of event at site beginning with 01". The first event at any site would be assigned an Event ID of "Year-01". Additional events at a particular site shall be numbered consecutively beginning with "Year-02". Even if a site isn't monitored until the latter half of the monitoring season (e.g., Site X is monitored during Feb. – Apr. 2002 – the latter half of the 2001-2002 monitoring season), its Event IDs include the year in which the general Caltrans monitoring season began (e.g., samples collected during the first event at Site X in Feb. 2002 are assigned Event IDs of "2001-01").

2.2. Monitoring Site ID

Field Type: Text
Field Size: 8 characters
Example: 7-21
Definition: A unique identification number assigned to the monitoring site by Caltrans.
Notes: The first part of the Monitoring Site ID corresponds to the Caltrans district in which the site is located.

2.3. Event Type

Field Type: Text
Field Size: 10 characters
Example: Non-storm
Definition: Describes if sampling event is associated with a storm event or a non-storm event.
Notes: Standardized Entry. Select value from standard list or contact database manager. This data field quickly describes whether an event record includes precipitation and flow data (i.e., event is a storm event) or does notnecessarily include such precipitation and flow data (i.e., event is a non-stormevent, such as a dry weather or groundwatersampling event; note: flow data could be provided for a dry weather event). Storm event records that are missing various precipitation and flow data due to malfunctioning equipment must be described with Event Type equal to "Storm" and include remarks in their Event Notes data field as to why certain data are missing.

<u>Standard List</u>

- Storm
- Non-storm

2.4. Rain Start Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which precipitation event begins.
Notes: Formatted as mm/dd/yyyy. See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.5. Rain Start Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which precipitation event begins.
Notes: Formatted for 24-hour clock (hh:mm). See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.6. Rain End Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which precipitation event ends.
Notes: Formatted as mm/dd/yyyy. See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.7. Rain End Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which precipitation event ends.
Notes: Formatted for 24-hour clock (hh:mm). See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.8. Event Rain (mm)

Field Type: Numeric
Field Size: Provide number only to three (3) decimal places.
Example: 15.989
Definition: Total rain at site for an event in millimeters (mm).
Notes: The Site Descriptions data field named Rain Record Source identifies the source of the Event Rain data for a particular monitoring site. See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.9. Max Intensity (mm/hr)

Field Type: Numeric
Field Size: Provide number only to three (3) decimal places.
Example: 12.183
Definition: Peak 5-minute intensity of a storm event in millimeters/hour (mm/hr).
Notes: Maximum Intensity is calculated as twelve times (12 x) the maximum rainfall recorded in any 5-minute period.

2.10. Antecedent Dry (days)

Field Type: Numeric
Field Size: Provide number only to one (1) decimal place.
Example: 12.0
Definition: Days since the end of the most recent prior Caltrans-defined precipitation event (whether monitored or not). If datum exists, irrespective of whether the prior event was monitored, data reporter should include Antecedent Dry datum as part of the Event Record for the current event.
Notes: See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.11. Antecedent Event Rain (mm)

Field Type: Numeric
Field Size: Provide number only to three (3) decimal places.
Example: 24.365
Definition: Total rain at site forthe most recent antecedent precipitation event in millimeters (mm). The antecedent precipitation event used for this data field should be the <u>same</u> antecedent precipitation event referenced in the Antecedent Dry data field. The quantification of Antecedent Event Rain does <u>not</u> include "inter-event" precipitation.
Notes: Using the examples provided above for Antecedent Dry and Antecedent Event Rain, the antecedent or prior precipitation event for Site X occurred 12.0 days ago, and delivered 24.37 mm of rainfall. The antecedent precipitation event must meet Caltrans' criteria for a precipitation event. See "Definition of a Precipitation Event" in the General Instructions section of this document.

2.12. Peak Capture

Field Type: Text
Field Size: 8 characters
Example: Y
Definition: Indication of whether peak flow was captured during sampling event.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- Y - Yes, peak flow was captured.
- N - No, peak flow was not captured.

2.13. Runoff Start Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which first runoff begins.
Notes: Formatted as mm/dd/yyyy.

2.14. Runoff Start Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which first runoff begins.
Notes: Formatted for 24-hour clock (hh:mm).

2.15. Runoff End Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which last runoff ceases.
Notes: Formatted as mm/dd/yyyy.

2.16. Runoff End Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which last runoff ceases.
Notes: Formatted for 24-hour clock (hh:mm)

2.17. Total Flow Volume (L)

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 38686
Definition: Total measured flow volume in liters (L) at a monitoring site for an event.

2.18. Peak Flow (L/s)

Field Type: Numeric
Field Size: Provide number only to two (2) decimal places.
Example: 3.40
Definition: Estimated peak flow of runoff in liters per second (L/s) at a monitoring site for an event.

2.19. Estimated Percent Capture

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 99
Definition: Estimated percentage of total event flow volume during which monitoring was performed (i.e., while the sampler was activated and flow-proportioned sample collection was successfully occurring). Report datum only if estimate is based on actual runoff and sample collection data measured by an instrument located at the monitoring site in question.
Notes: Periods of sampler malfunction or failure to collect sample must be excluded from the "captured flow".

2.20. Cumulative Precipitation (mm)

Field Type: Numeric
Field Size: Provide number only to three (3) decimal places.
Example: 100.761
Definition: Estimated cumulative precipitation in millimeters (mm) at monitoring site since the beginning of the water year (October 1), up to the Rain Start Date/Time for the monitored event. All precipitation that has fallen to date at a site, including "inter-event" precipitation, should be included in the Cumulative Precipitation value reported for that site for a particular event.

2.21. Sample Collector

Field Type: Text
Field Size: 25 characters
Example: Peggie Sampson
Definition: Name of the individual who collected the sample.

2.22. Organization

Field Type: Text
Field Size: 30 characters
Example: Acme Environmental
Definition: Organization to which the sample collector is associated.

2.23. Event Notes

Field Type: Text
Field Size: 255 characters
Example: Runoff End Time missing due to malfunctioning flow meter.
Definition: This data field contains any notes or remarks about the sampling event or various data fields used to describe the sampling event.
Notes: Data reporter should provide a note for any data field left blank. It is not appropriate to provide a note in a particular cell of a data field other than Event Notes. Considering the example above, it is not appropriate to place the note, "Runoff End Time missing due to malfunctioning flow meter", in the Runoff End Time data field. The note must be entered into the Event Notes data field.

3. WATER QUALITY SITE DESCRIPTIONS

3.1. Monitoring Site ID

Field Type: Text
Field Size: 8 characters
Example: 7-21
Definition: A unique identification number assigned to the monitoring site by Caltrans.
Notes: The first part of the Monitoring Site ID corresponds to the Caltrans district in which the site is located.

3.2. Monitoring Year

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 2001
Definition: Identifies the monitoring season a site is monitored by populating the data field with the year the current monitoring season begins.
Notes: In regard to the 2001-2002 monitoring season, the Monitoring Year value for each site monitored would be "2001" – the year the current monitoring season began. The term Monitoring Year refers to only a single calendar year, while the term Monitoring Season necessarily refers to two calendar years, such as 2001-2002.

3.3. Site Name

Field Type: Text
Field Size: 100 characters
Example: DICE Site 12/23
Definition: Name of monitoring site.
Notes: Site Name is meant to be a shorthand designation for the monitoring site, and the "common name" by which the sampling crew and data reporter refer to the site.

3.4. Site Description

Field Type: Text
Field Size: 255 characters
Example: East of Ocean View Avenue Onramp to EB 210 Fwy; a low-density residential site within foothills.
Definition: Describes monitoring site in some detail, including observations related to site's location and physical characteristics.
Notes: In addition to the above definition, this field can be used to describe changes observed at the site since the prior monitoring season.

3.5. Caltrans District

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 7
Definition: Caltrans district in which monitoring site is located.

3.6. Hydrologic Sub-Area

Field Type: Numeric
Field Size: Provide 5-digit number in the format XXX.XX
Example: 405.32
Definition: State Water Resources Control Board (SWRCB) Hydrologic Unit Sub-Area in which monitoring site is located.
Notes: SWRCB Hydrologic codes are 6-byte strings composed of numbers and a decimal point. The meaning associated with each byte position is shown below.

Byte(s)	Meaning
1	Hydrologic Region
2,3	Hydrologic Unit
4	always a decimal point
5	Hydrologic Area
6	Hydrologic Sub-Area

Hydrologic sub-areas are typically identified in Basin Plans. They may also be found at the Water Quality Standards Inventory Database web site: http://endeavor.des.ucdavis.edu/wqsid/region.asp

3.7. County

Field Type: Text
Field Size: 20 characters
Example: Los Angeles
Definition: County in which monitoring site is located.
Notes: It is not necessary to include the word "County" along with the actual name of the county in which the monitoring site is located.

3.8. RWQCB

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 4
Definition: Regional Water Quality Control Board (RWQCB) region in which monitoring site is located.

3.9. Runoff Characterization

Field Type: Text
Field Size: 15 characters
Example: Hwy
Definition: Type of runoff characterized by samples collected from monitoring site.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- Hwy - Highway or Freeway
- Maintenance - Maintenance facility
- Bridge - Bridge
- Parking - Parking lot (e.g., Park & Ride facility)
- Rest Area - Rest area
- A/D - Acceleration/Deceleration area
- Construction - Construction
- BMP - Site designed for storm water treatment
- Receiving - Receiving water (described further in "Receiving Water Type)
- Watershed - Municipal or rural watershed (described further in "Land Use")

3.10. Surface Type

Field Type: Text
Field Size: 25 characters
Example: Pavement
Definition: Describes the predominant surface type associated with a Caltrans facility.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- Landscape - Landscaping
- Pavement - Pavement only
- Right-of-Way - Right-of-Way (includes landscape and pavement)

3.11. Land Use

Field Type: Text
Field Size: 4 characters
Example: R
Definition: Predominant land use of the tributary catchment area. This field describes the predominant land use of the area from which sampled runoff originates.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- R - Residential
- C - Commercial
- I - Industrial
- A - Agricultural
- F - Forest
- O - Open
- T - Transportation Facility (e.g., bridge, county road, highway, etc.)
- M - Mixed (no predominant land use can be discerned)

3.12. Catchment Area (ha)

Field Type: Numeric
Field Size: Provide number only to two (2) decimal places.
Example: 2.91
Definition: Total surface area in hectares (ha) draining to the point of collection of the storm water runoff sample.

3.13. Impervious Fraction

Field Type: Numeric
Field Size: Provide number only to two (2) decimal places.
Example: 0.67
Definition: Estimated fraction of the catchment area that is effectively impervious.

3.14. BMP Type

Field Type: Text
Field Size: 35 characters
Example: EDB
Definition: Describes the type of BMP represented by the monitoring site. This data field to be populated only for monitoring sites designed to treat storm water runoff. All non-BMP sites should have this data field value reported as "N/A".
Notes: Standardized Entry. Select value from standard list or contact database manager.

<u>Standard List</u>

- Retention - Retention Pond
- Swale - Biofiltration Swale
- EDB - Extended Detention Basin
- Wet Pond - Wet Detention Pond
- Dry Pond - Extended Dry Detention Pond
- Compost - Compost Media Filter
- Zeolite - Zeolite Media Filter
- SF - Sand Media Filter
- IB - Infiltration Basin
- IT - Infiltration Trench
- TCB - Trapping Catch Basin
- Insert - Drain Inlet Insert
- Strip - Biofiltration Strip
- Strip-Trench - Filtration Strip and Infiltration Trench Treatment Train
- OWS - Oil/Water or Debris Separator
- MCTT - Mult-chambered Treatment Train
- CDS - Continuous Deflector Separator
- DIC - Drain Inlet Cleaning
- Pickup - Litter Pickup (litter studies)
- Sweep - Street Sweeping (litter studies)
- ModifyIn - Modified Inlet (litter studies)
- Grate - Bicycle Grate/LID (litter studies)
- ModGrate - Modified Bicycle Grate (litter studies)

Alternate Value: - N/A (see Definition above).

3.15. Point of Collection

Field Type: Text
Field Size: 12 characters
Example: Discharge
Definition: Describes the point of sample collection at the monitoring site.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- Discharge - Outfall to receiving water or outlet to conveyance system
- Inlet - Inlet to BMP
- Outlet - Outlet to BMP
- Within - Somewhere within BMP
- Bypass
- Overflow
- Overland - Overland flow
- Receiving - Location(s) in receiving water (stream, lake, etc.)

3.16. Control Site

Field Type: Text
Field Size: 20 characters
Example: 7-156
Definition: The Caltrans Monitoring Site ID for the control or BMP "inlet" site associated with the BMP "outlet" or BMP "within" site in question. All non-BMP sites and BMP "inlet" sites should have this data field value reported as "N/A".
Notes: A small number of BMP sites do not possess a control or reference site. These sites should have this data field populated with the value "No Control for BMP".

Alternate Values: - N/A (see Definition above).
- No Control for BMP (see Notes above).

3.17. Cut-Fill

Field Type: Text
Field Size: 15 characters
Example: Cut
Definition: Identifies the roadway or facility as a cut, fill, grade or hybrid type (e.g., Grade/Fill) site.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- Cut - Sample collected from a cut area.
- Fill - Sample collected from a fill area.
- Grade - Sample collected from a grade area.
- Cut/Fill - Sample collected from a roadway or facility with both cut and fill areas.
- Grade/Cut - Sample collected from a roadway or facility with both grade and cut areas.
- Grade/Fill - Sample collected from a roadway or facility with both grade and fill areas.

3.18. Roadway Post Mile

Field Type: Text
Field Size: 18 characters
Example: I-210-EB-18.45
Definition: A four-part descriptor that describes the roadway type, roadway number, traffic direction, and post mile of the Caltrans highway facility nearest to the monitoring site. If no such Post Mile exists for a site, the data field value should be reported as "N/A".
Notes: The Roadway Post Mile value is a compilation of the following four pieces of information: **Roadway Type-Roadway Number-Traffic Direction-Post Mile**
Standardized entries exist for roadway type and traffic direction.
Select values from standard lists or contact database manager.

Standard Lists

Roadway Type

- I - Interstate
- SR - State Route
- CR - County Road

Traffic Direction

- EB - Eastbound
- WB - Westbound
- NB - Northbound
- SB - Southbound

Alternate Value: - N/A (see Definition above).

3.19. Latitude

Field Type: Numeric
Field Size: Provide number to five (5) decimal places.
Example: 34.20969
Definition: Latitude of monitoring site in decimal degrees to five (5) decimal places.
Notes: Data should be collected using a differential GPS instrument with sufficient accuracy to report degrees to five decimal places.

3.20. Longitude

Field Type: Numeric
Field Size: Provide number to five (5) decimal places.
Example: 118.22469
Definition: Longitude of monitoring site in decimal degrees to five (5) decimal places.
Notes: Data should be collected using a differential GPS instrument with sufficient accuracy to report degrees to five decimal places

3.21. Datum

Field Type: Text
Field Size: 20 characters
Example: NAD 83
Definition: Datum by which latitude and longitude reported.
Notes: NAD 27 Conus is the preferred datum.

3.22. Rain Record Source

Field Type: Text
Field Size: 50 characters
Example: NOAA
Definition: Source of precipitation record used in precipitation estimates.
Notes: Report "Onsite" if rain gauge is located at the monitoring site, or report the name/location of the nearest rain gauge. Actual precipitation event record (i.e., rainfall data from rain gauge) should be included in a Time Series worksheet for the event (see Time Series data field description in this section).

3.23. Receiving Water Type

Field Type: Text
Field Size: 20 characters
Example: Intermittent
Definition: Type of receiving water sampled or into which storm water runoff is discharged.
Notes: Standardized Entry. Select value from standard list or contact database manager.

Standard List

- Intermittent - Intermittent or seasonal stream or channel
- Wetlands - Seasonal or perennial wetlands
- River - Perennial freshwater river
- Stream - Perennial freshwater stream
- Lake - Freshwater lake or impoundment
- Pond - Freshwater pond
- Bay - Salt or brackish water bay
- Estuary - Salt or brackish water estuary
- Ocean
- Onsite Infiltration - Runoff percolates into soil profile surrounding monitoring site

3.24. AADT

Field Type: Numeric
Field Size: n/a
Example: 120000
Definition: Average annual daily traffic (AADT) flow at or near monitoring site.
Notes: If site is a maintenance yard, parking lot, or rest area, report the estimated number of vehicles entering and leaving the facility each day.

3.25. AADT Source

Field Type: Text
Field Size: 100 characters
Example: http://www.dot.ca.gov/hq/traffops/saferesr/trafdata/1999all.htm
Definition: Source or reference for reported AADT value.
Notes: A searchable AADT database is available at the Caltrans Traffic and Vehicle Data Systems Unit web page:
http://www.dot.ca.gov/hq/traffops/saferesr/trafdata/

3.26. Total Lanes

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 4
Definition: The total number of traffic lanes (moving in both directions) of the Caltrans highway facility nearest to the monitoring site.

3.27. Time Series

Field Type: Text
Field Size: 50 characters
Example: Site 7-21 Time Series_2001-2002.xls
Definition: The name of the Excel worksheet in which time series data (flow, precipitation, etc.) collected at a monitoring site is submitted.
Notes: Times series data collected during storm events at a monitoring site can be submitted as multiple worksheets (one worksheet for each storm event) in a single workbook that represents all times series data collected at that site during an entire monitoring season. The Excel workbook file should be labeled with the Caltrans Monitoring Site ID and monitoring season (e.g., 2001-2002 or 01-02) to which it corresponds.

3.28. Map

Field Type: Text
Field Size: 50 characters
Example: Site 7-21 Map_2001-2002.pdf
Definition: The name of the electronic document containing a map that shows the location of the monitoring site.
Notes: The actual electronic document containing a map of the monitoring site should be submitted along with the rest of the data collected for the site, including water quality data, time series data, and one or more photographs. The map should contain the clearly marked location of the monitoring site, latitude and longitude markers, and the watershed contributing flows to the site. The electronic map document should be labeled with the Caltrans Monitoring Site ID and monitoring season (e.g., 2001-2002 or 01-02) to which it corresponds.

3.29. Photograph

Field Type: Text
Field Size: 50 characters
Example: Site 7-21 Photo_2001-2002.jpeg
Definition: The name of the electronic document containing photograph(s) of the monitoring site.
Notes: The actual electronic document containing photograph(s) of the monitoring site should be submitted along with the rest of the data collected for the site, including water quality data, time series data, and a map. The electronic photograph document should be labeled with the Caltrans Monitoring Site ID and monitoring season (e.g., 2001-2002 or 01-02) to which it corresponds.

3.30. Site Notes

Field Type:	Text
Field Size:	255 characters
Example:	No AADT value provided for this site. Site is located along a county road for which no AADT data are available.
Definition:	This data field contains any notes or remarks about the monitoring site or various data fields used to describe the monitoring site.
Notes:	Data reporter should provide a note for any data field left blank. It is not appropriate to provide a note in a particular cell of a data field other than Site Notes. Considering the example above, it is not appropriate to place the note, "No AADT value provided for this site. Site is located along a county road for which no AADT data are available", in the AADT data field. The note must be entered into the Site Notes data field.

4. AQUATIC TOXICITY SAMPLE DESCRIPTIONS

4.1 Event ID

Field Type: Text
Field Size: 8 characters
Example: 2001-01
Definition: A unique, site-specific identification number assigned to a sampling event.
Notes: Event ID follows the format "Year monitoring season begins-Number of event at site beginning with 01". The first event at any site would be assigned an Event ID of "Year-01". Additional events at a particular site shall be numbered consecutively beginning with "Year-02". Even if a site isn't monitored until the latter half of the monitoring season (e.g., Site X is monitored during Feb. – Apr. 2002 – the latter half of the 2001-2002 monitoring season), its Event IDs include the year in which the general Caltrans monitoring season began (e.g., samples collected during the first event at Site X in Feb. 2002 are assigned Event IDs of "2001-01").

4.2 Monitoring Site ID

Field Type: Text
Field Size: 8 characters
Example: 7-21
Definition: A unique identification number assigned to the monitoring site by Caltrans.
Notes: The first part of the Monitoring Site ID corresponds to the Caltrans district in which the site is located.

4.3 Field Sample ID

Field Type: Text
Field Size: 35 characters
Example: 134-0010-281500A
Definition: Field identification number assigned to sample/sample vessel.
Notes: This data field can contain any combination of text and numeric characters.

4.4 Sample Start Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which composite sampling begins or grab sample is collected. In regard to composite samples, specifically the date on which the sampler was activated <u>and</u> the flow meter began totalizing flow.
Notes: Formatted as mm/dd/yyyy.

4.5 Sample Start Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which composite sampling begins or grab sample is collected. In regard to composite sampling, specifically the time at which the sampler was activated and the flow meter began totalizing flow.
Notes: Formatted for 24-hour clock (hh:mm).

4.6 Sample End Date

Field Type: Date
Field Size: n/a
Example: 11/05/2001
Definition: Date on which composite sampling ends. Specifically, the date on which the sampler was de-activated and the flow meter stopped totalizing flow.
Notes: Formatted as mm/dd/yyyy.

4.7 Sample End Time

Field Type: Time
Field Size: n/a
Example: 15:24
Definition: Time at which composite sampling ends. Specifically, the time at which the sampler was de-activated and the flow meter stopped totalizing flow.
Notes: Formatted for 24-hour clock (hh:mm).

4.8 Sample Source

Field Type: Text
Field Size: 12 characters
Example: Storm
Definition: Identifies the source of the water quality sample analyzed.
Notes: Standardized Entry. Enter value from standard list or contact database manager.

Standard List

- Storm - Sample from storm water runoff
- Rain - Sample from rainwater
- Receiving - Sample from receiving water
- Vadose - Sample from vadose zone
- Base - Sample from dry-weather base flow
- Groundwater - Sample from groundwater
- Non-storm - Sample from non-storm water runoff
- BMP media - Sample from material used to construct BMP device

4.9 Event Representation

Field Type: Text
Field Size: 12 characters
Example: FF
Definition: Describes the part of the event represented by the sample.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- FF - Event first flush
- Peak - Event peak flow
- Whole - Whole storm
- Discrete - One or more discrete samples collected during an event that cannot be described as representing the first flush, peak or whole storm portion of the event. This value will typically, although not exclusively, be used for a "Non-storm" Sample Source.
- X-hr Comp - Used to describe a planned composite sampling event lasting X hours.

4.10 Sample Type

Field Type: Text
Field Size: 2 characters
Example: C
Definition: Describes the way in which sample was collected.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- C - Composite Sample: A sample made up of multiple sub-samples (aliquots) collected over some spatial or temporal scale.
- G - Grab Sample: A single sample that represents conditions at a distinct point in time.

4.11 Sample Matrix

Field Type: Text
Field Size: 10 characters
Example: Water
Definition: Matrix from which sample was analyzed.
Notes: Standardized Entry. Enter value from standard list or contact database manager.
Standard List

- Water
- Sediment (not currently included in Caltrans toxicity protocols).

4.12 Collection Method

Field Type: Text
Field Size: 6 characters
Example: Auto
Definition: Describes the way in which a sample was collected.
Notes: Standardized Entry. Select value from standard list or contact database manager.
Standard List

- Auto - Sample aliquot collected by automated sampling device.
- Manual - Sample aliquot collected manually.

4.13 Treatment

Field Type: Text
Field Size: 30 characters
Example: 100 ppb PBO
Definition: Description of any treatment (manipulation or chemical addition) to the original runoff sample.
Notes: This data field is only to include simple and/or targeted manipulations of the sample (chemical spikes, aeration, pH adjustments, etc.) and is not intended to incorporate all sample manipulations that may be included in a full TIE.

4.14 Test Type

Field Type: Text
Field Size: 18 characters
Example: Acute, screen
Definition: Designates if the test was an acute or chronic test and if the test was a screening level test or a full dilution series test.
Notes: Standardized entry.
Standard List

- Acute, screen
- Acute, dilutions
- Chronic, screen
- Chronic, dilutions

4.15 Test Start Date

Field Type: Date
Field Size: n/a
Example: 02/22/2001
Definition: Date on which the toxicity test is initiated.
Notes: Formatted as mm/dd/yyyy

4.16 Test Start Time

Field Type: Time
Field Size: n/a
Example: 14:25
Definition: Time at which toxicity test is initiated.
Notes: Formatted for 24-hour clock (hh:mm)

4.17 Test End Date

Field Type: Date
Field Size: n/a
Example: 02/24/01
Definition: Date toxicity test is completed.
Notes: Formatted as mm/dd/yyyy

4.18 Test End Time

Field Type: Time
Field Size: n/a
Example: 14:30
Definition: Time at which test is terminated.
Notes: Formatted for 24-hour clock (hh:mm)

4.19 Test Organism

Field Type: Text
Field Size: 25 characters
Example: *Ceriodaphnia dubia*
Definition: Identify species tested.
Notes: Use scientific name (e.g. *Pimephales promelas* for fathead minnow)

Standard List

- *Ceriodaphnia dubia*
- *Pimephales promelas*
- *Selenastrum capricornutum (changed to Raphidocelis subcapitata)*
- *Oncorhynchus mykiss*

4.20 Toxicity Endpoint

Field Type: Text
Field Size: 20 characters
Examples: Mortality
Definition: Provide the endpoint tested in a given toxicity test.
Notes: Standardized entry, see standard list. Use endpoints associated with the appropriate species and test type as indicated in Table 1 of the general instructions section. Consideration of screening level or dilution series tests is not required for this data field.

Standard List

- Mortality
- Reproduction
- Growth
- Growth (cell number)

4.21 Result Type

Field Type: Text
Field Size: 12 characters
Examples: Significance
Definition: Expression of the endpoint measured.
Notes: Standardized entry, see standard list. Use result types associated with the appropriate endpoints and test type as indicated in Table 1 of the general instructions section. Note that screening level and dilution series tests will provide different result types as indicated in the standard list.

Standard List

- Mean mortality
- Statistically significant difference from control
- Standard error
- LC50
- LC25
- LC10
- EC50
- EC25
- EC10
- IC50
- IC25
- IC10
- NOEC
- LOEC
- Chronic Toxic Units
- Mean reproduction
- Mean growth

4.22 Units

Field Type: Text
Field Size: 15 characters
Example: %
Definition: Describes units of reported result.
Notes: Standardized entry, see list below. Also see Table 1.

Standard List	Example
• %	-percent mortality
• yes/no	-statistical significant difference (answer either yes or no)
• neonates/adult	-C.dubia reproduction
• mg/individual	-growth
• cells/mL	-algal growth
• % dilution	-only used if a dilution series was run
• TUa	-acute toxic unit= LC50, etc. divided by the highest tested concentration
• TUc	-chronic toxic unit=NOEC, etc. divided by highest tested concentration

4.23 Numerical Qualifier

Field Type: Text
Field Size: 1 character
Example: <
Definition: An indication of what the value represents (i.e. a minimum, maximum, or "exact" value).
Notes: Standardized entry. Enter name from standard list or notify database manager.

Standard List

- < -actual value is less than the reported value (non-detect) ADD
- = -value is as reported.
- > -actual value is greater than reported value.

4.24 Result

Field Type: Numeric
Field Size: n/a
Example: 30
Definition: Reported test result (30% mortality).
Notes: Standardized entry.

4.25 Statistical Significance (Statsig)

Field Type: Text
Field Size: 3 characters
Example: Yes
Definition: To indicated if there was a significant difference between the runoff sample result and the control result.
Notes: Standardized entry, see standard list.

Standard List

- Yes
- No

4.26 Test Day

Field Type: Numeric
Field Size: Provide number as an integer with zero (0) decimal places.
Example: 7
Definition: Indicates the day of a multi-day test. For example, the first 24 hours (0-24 hours) of the test is referred to as day 1, 24-48 hours as day 2, etc.
Notes: Formatted as a whole number, do not use hours (48 hrs = 2 days).

4.27 TIE Performed

Field Type: Text
Field Size: 3 characters
Example: Yes
Definition: Indicates if TIE manipulations are associated with this sample.
Notes: Standardized entry, see standard list

Standard List

- Yes
- No

4.28 Suspect Toxicant

Field Type: Text
Field Size: 50 characters
Example: Diazinon
Definition: Indicates if a specific toxicant or toxicants were identified using TIE methods.
Notes: List constituent(s) identified as toxicant(s). List class of compound (metals) if specific constituent was not identified. If neither the class nor specific constituent were identified list "none". See standard list for compound classes.

Standard List

- Metals
- Non-polar organics
- Volatiles
- Oxidants
- pH dependent
- Polar organics
- Anionic inorganic

4.29 TIE Phase completed

Field Type: Numeric
Field Size: 1 characters
Example: 2
Definition: TIEs are conducted in a series of phases, 1-3. This data field identifies which phases were completed.
Notes: Standardized entry. See standard list.

Standard List

- 1
- 2
- 3

4.30 Control ID

Field Type: Text
Field Size: 35 characters
Example: Lab-001-QA
Definition: Identifies the laboratory control associated with the sample.
Notes: Standardized entry.

4.31 Lab Name

Field Type: Text
Field Size: 25 characters
Example: APPL
Definition: Name of laboratory that analyzed sample.
Notes: Standardized Entry. Select value from standard list. The list of analytical laboratories used by the Caltrans Storm Water Management Program will likely change over time, and therefore the Standard List below might not include the name of every laboratory used in any given monitoring year.

Standard List

- Aquascience
- Aquatic Bioassay and Consulting (ABC) Laboratory
- Associated Labs
- BSK
- CEL - Calscience Environmental Labs
- Del Mar Analytic
- GeoAnalytical Labs
- North Coast Labs
- Pat-Chem
- Quanterra
- Silliker
- Soil Control Lab
- ToxScan
- UCDATL -UC Davis Aquatic Toxicity Lab

4.32 Lab Sample ID

Field Type: Text
Field Size: 25 characters
Example: 120085-400A
Definition: Laboratory identification number assigned to sample by analytical laboratory.
Notes: This data field can contain any combination of text and numeric characters.

4.33 Contract Number

Field Type: Text
Field Size: 12 characters
Example: 43A005
Definition: Caltrans contract number under which sample was collected.
Notes: This data field can contain any combination of text and numeric characters.

4.34 Task Order Number

Field Type: Text
Field Size: 6 characters
Example: 11
Definition: Caltrans task order number under which sample was collected.
Notes: This data field can contain any combination of text and numeric characters.

4.35 Toxicity Notes

Field Type: Text
Field Size: 255 characters
Example: Statistically significant toxicity was not observed, but sublethal effects (behavior or physical defects) were observed.
Definition: This data field contains any notes or remarks about the toxicity sample or various data fields used to describe the toxicity sample.
Notes: Data reporter should provide a note for any data field left blank (with the exception of Overall Qualifier and Statistical Significance). It is not appropriate to provide a note in a particular cell of a data field other than Toxicity Notes. Considering the example above, it is not appropriate to place the note, "Sublethal effects including changes in behavior and physical defects were observed", in the Result Type data field. The note must be entered into the Toxcity Notes data field.