Quality Assurance Project Plan

FOR

SURVEY OF TOXIC ALGAE FROM THE SALTON SEA

Principal Investigator: D. John Faulkner
Institution: Scripps Institution of Oceanography, UCSD

Section A: PROJECT MANAGEMENT

A1 Project Title and Approval Sheet

Survey of Toxic Algae from the Salton Sea

Dr. D. John Faulkner
Scripps Institution of Oceanography, University of California at San Diego

Technical Project Manager	_____	_____
	D. John Faulkner	Date
Salton Sea Project Manager	_____	_____
	Tom Kirk	Date
Salton Sea QA Coordinator	_____	_____
	Barry Gump	Date

A2 Table of Contents

A3 Distribution List

Salton Sea Authiority

A4 Project/Task Organization and Responsibilities (organizational chart)

The project is part of a study entitled "An Environmental Reconnaissance of the Salton Sea". The project was initiated by the Salton Sea Research Management Committee and is under the direction of the Salton Sea Authority. The project reports through the study Project Officer to the Executive Director of the subcommittee.

Samples of algae will be collected by Kris Reifel, under the direction of Dr. Stuart Hurlbert, and will be delivered to us as frozen specimens. Under the supervision of Dr. Faulkner, Mike McCoy will prepare algal extracts that will be assayed in house by Mary Kay Harper and sent to Dr. Tony Rocke at National Wildlife Health Center for toxicity screening. Samples will be accompanied by "action sheets". Each specimen will be subjected to limited non-destructive spectroscopic analysis before being forwarded. Progress will be monitored by the Project officer, Milton Friend.

A5 Project Definition and Background:

The overall goal of this project is to test the hypothesis that algal toxins contribute to bird deaths at the Salton Sea. The immediate goal is to prepare extracts of phytoplankton specimens collected by researchers at San Diego State University and determine their toxicity against brine shrimp and a standard suite of microorganisms. The extracts will be screened in mice by toxicologists at the National Wildlife Heath Center.

Toxic extracts will be fractionated to define the polarity and chemical characteristics of the toxin(s). Every effort will be made to identify known toxins but the chemical characterization of unknown toxins will probably be beyond the scope of this survey. The invertebrates on which the birds feed will also be sampled to determine whether they are concentrating algal toxins. Given the time restraints of this survey, it will not be proctical to investigate the role of the bacterial loop in the Salton Sea food chain.

A6 Project/Task Description:

PROJECT TASK LIST AND TIMELINE

Task No.	Task	Responsibility	Start Date	Completion Date

1	Preparation of extracts	McCoy	1 Jan 99	30 Dec 99
2	In house screening	Harper	1 Jan 99	30 Dec 99

A7 Quality Objectives and Criteria for Measurement Data

Data to be collected:

Task 1 No data will be generated in this portion of the project.

Task 2 The toxicity of extracts toward brine shrimp *Artemia salina*. NMR spectra of all extracts.

Conditions under which data are to be collected:

Crude extracts are screened in the standard brine shrimp assay (Solis, P.N.; Wright, C.W.; Anderson, M.M.; Gupta, M.P.; Phillipson, J.D. *Planta Med.* **1993**, *59*, 250-252) at 400, 200, 100 and 50 µg/mL.

NMR spectra are recorded on Varian Inova 300 MHz, Varian Gemini 400 MHz and Varian Unity 500 MHz spectrometers.

Measurement Performance Criteria

Parameter	Detection Limit	Reporting Limit	Estimated Accuracy	Accuracy Protocol*	Estimated Precision	Precision Protocol**

DATA COMPLETENESS

Parameter	Number of Valid Sample Results for Data Management Purposes	Number of Valid Samples Collected and Analyzed	Percent Complete

Data Representativeness:

The data will provide a qualitative assessment of the presence of toxic algal blooms in the Salton Sea. The sampling strategy and its effectiveness will be addressed by the San Diego State research program.

Data Comparability:

Not applicable
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A8 Special Training Requirements/Certification

None

A9 Documentation and Records

A separate laboratory notebook will be used to record all data from this project. Action sheets will be generated for each sample. The action sheet will record the date and collecting location of the sample, the composition of the sample, if known, the dry

weight of the sample, the weights of each extract derived from the sample and the brine shrimp toxicity data. A copy of the action sheet will accompany every set of extracts forwarded to National Wildlife Health Center.

Section B: MEASUREMENT/DATA ACQUISITION

B1 Sampling Process Design (Experimental Design)

Sampling Design, Assumptions and Rationale:
Kris Reifel in Dr. Hurlebert's group at San Diego State University is responsible for obtaining algal specimens. Our responsibilities do not include any tasks that involve sampling
Procedures for Locating and Selecting Environmental Samples:
N/A

Site Location	Sample Matrix	Sampling Stations per Site	No. of Samples per Station	Sampling Method	Sample Type	Sample Parameters	Frequency of Sampling	QC Samples

Schedule for Project Sampling Activities: N/A

Classification of Measurements as Critical or Noncritical
Validation of Any Nonstandard Methods

B2 Sampling Method Requirements

Sampling Methods:

Types of Samples to be Collected:

Sampling Method's Requirements:

Decontamination Procedures and Materials:
All solvents used for extraction and partition of samples are redistilled immediately prior to use. Chemicals use in this project are Analar Grade or equivalent quality.

Sampling System Failure Response and Corrective Action Process:

B3 Sample Handling and Custody Requirements

PARAMETER TABLE

Parameter	Number of Samples	Matrix	Sample Preservation	Holding Time

Custody Procedures: All samples are maintained at -20 C. All samples are treated as toxic until shown to be non-toxic.

B4 Analytical Methods Requirements

Sample Parameter	Matrix	Analytical Method Reference*

*If any methods used are not EPA or State certified lab standard methods, then provide a descriptive paragraph describing each of those methods. Field analytical procedures can also be entered into this table.

Data's ability to meet the QC acceptance criteria:

^{1}H NMR chemical shift data are reported to 0.01 ppm: the accuracy and reproducibility of these measurements are probably ± 0.02 ppm but the accuracy required for interpretation is ± 0.1 ppm. ^{13}C NMR chemical shift data are reported to 0.1 ppm: the accuracy and reproducibility of these measurements are probably ± 0.1 ppm but the accuracy required for interpretation is ± 1 ppm.

The brine shrimp assay is generally regarded as a yes/no assay for extracts and is only quantified for pure compounds. We classify extracts as mildly toxic if >80% of the brine shrimp die at the highest concentration screened and toxic if 100% of the brine shrimp die at the lowest concentration screened. The negative control should show less than 25% death or the screen is repeated.

Sample preparation procedures:

Aliquots of the extracts are weighed on a Mettler AE240 balance and are diluted to obtain standard solutions of 400 μg/mL, which are then subject to serial dilution.
For NMR spectroscopy, methanol extracts are dissolved in CD_3OD (>99%), ethyl acetate extracts are dissolved in $CDCl_3$ (>98%) and aqueous extracts are dissolved in D_2O (>99%) to prepare solutions containing, ideally, 40 mg dry wt./mL.

Deviations or selection of method options:

B5 Quality Control Requirements

QC Procedures:

NMR spectra are calibrated using the solvent peak as an internal calibration.

The brine shrimp assay cannot easily be calibrated when used to determine the presence of toxins in crude extracts. It requires an experienced researcher to follow the assay visually and detect anomolies. Mary Kay Harper has seven years of experience and been able to use the assay even under the most primitive conditions at sea where a balance is unavailable.

Field QC checks

Laboratory QC checks

Corrective actions:

B6 Instrument/Equipment Testing, Inspection, and Maintenance Requirements

The NMR spectrometers are maintained by the SIO Analytical Facility, who perform routine maintenance whenever an instrument fails to meet specifications.

B7 Instrument Calibration and Frequency

Balances are cleaned and calibrated yearly. NMR spectrometers use internal calibration and are tuned for each sample. A standard solution of menthol is used to calibrate line widths in the event that the instrument has to be retuned after a power failure.

B8 Inspection/Acceptance Requirements for Supplies and Consumables

Supplies are logged under a cradle-to-grave tracking system administered by UCSD's Office of Enviromental Health and Safety. Each chemical is bar-coded.

B9 Data Acquisition Requirements (Non-direct Measurements)

Identification of toxins requires extensive use of the literature. We have copies of most relevant papers on marine natural products, including toxins, and these data form the basis the database of Marine Natural Products. Authentic specimens of marine toxins can often be obtained from other researchers and authenticated spectral data can be obtained from supplemental material in papers or from the authors. The Scripps Institution of Oceanography has an excellent library and can obtain copies of any paper in the literature.

B10 Data Management

Collection data are kept by Kris Reifel and are forwarded to us electronically. These data are transferred to an Action Sheet (see attached) for each specimen. Each step in the extraction and screening process is recorded with the relevant dates and weights. When extracts are sent to NWHL, a copy of the action sheet will accompany the specimen and a second copy will be forwarded electronically.

Brine Shrimp assay data will be recorded in a laboratory notebook and an interpretation recorded on the action sheet.

Printed NMR spectral data, and any other printed data recorded for crude extracts, will be filed in 3-ring binders. The original unprocessed NMR data will be stored electronically on quarter inch tape. Interpretations of NMR data will be recorded on the action sheets.

The action sheets are filed as paper copies also and stored on electronic media which will be backed up when needed, at least once a month.

Section C: ASSESSMENT/OVERSIGHT

C1 Assessments and Response Actions

C2 Reports to Management

Section D: DATA VALIDATION AND USABILITY

D1 Data Review, Validation, and Verification Requirements

D2 Validation and Verification Methods

D3 Reconciliation with Data Quality Objectives