1. Procedure summary

The purpose of this SOP is to outline the procedures to follow when performing the preview tube bioassay to assess the potential risks and efficacy of a pesticide application or other change in media composition.

1.1. Related Procedures

Safe Use of Pesticides	LC-03-009-002

1.2. Procedure impacts and concerns

Safety	(1) All pesticides will be managed in accordance with the New Mexico Department of Agriculture Pesticide Compliance Section. http://nmdaweb.nmsu.edu/pesticides. The EPA Worker Protection Standard can be used as an additional reference: http://www.epa.gov/pesticides/safety/workers/PART170.htm	
Quality	Crop Protection team: Review procedures with Site Manager to ensure understanding and compliance. Prior to pesticide handling, ensure staff has current State of New Mexico pesticide applicators licenses, HAZCOM training, and quarterly compliance refresher training. Ensure that each employee has complete knowledge on PPE requirements, use, limitations, care, maintenance, and disposal.	
Delivery	Lab Staff: Handle and apply pesticides in accordance with the SOP. Participate in required trainings.	
Environmental		
Cost	NA	
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required. See 29 CFR 1910.120 and 1200. An AUL list, MSDSs and label information will be available for easy reference in a binder next to the pesticide storage cabinets, but outside the spill containment area, and in the administration building	Site Manager: Conduct general pesticide safety awareness training for all workers. For all pesticides, maintain current Material Safety Data Sheets (MSDS), label information, and a current Authorized Use List (AUL). Perform monthly inspections of the entire site, checking PPE, pesticide storage, pesticide signage, etc.

1.3. Responsibilities and owners

Document Owner	Manage content and distribution	Kalli Lambeth
Process Owner	Responsible for content and process validation	Alina Corcoran
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

This process describes how to perform the Preview Tube bioassay to evaluate the efficacy and toxicity of a potential or planned chemical/pesticide application to field culture. Gradients of chemicals/pesticides are evaluated against a control to determine the best field application for maintenance of a healthy culture.

Site environmental use permits for these pesticides on file with site manager

2.2. Process steps

2.3.1 Prerequisite SOP

2.3.1.1 Any employee involved in performing preview tube assays is to familiarize themselves with SOP LC-03-009-002 'Safe Use of Pesticides' for all information concerning: storage, certification, handling, spills, first aid and transport.

2.3.2 Experimental Set-up

2.3.2.1 For the control and each chemical treatment to be applied to the culture, place three 10mL culture tubes (Fisher cat #14-956-1J) into an 18-tube rack.

2.3.2.2 Distribute 5mL pond culture to each tube with a serological pipette or pipetter.

2.3.2.3 Label the chemical gradients, triplicate designations (A, B, C), date, and nature of the experiment on tubes and/or rack.

2.3.2.4 Dilute chemical/pesticides to a 1000ppm stock concentration.

2.3.2.5 Add chemical/pesticide to each tube using a pipetter.

2.3.2.6 Take triplicate OD and fluorescence readings on each tube. Refer to SOP LC-07-002-001 and LC-07-002-003.

2.3.2.7 Place tube racks in acrylic CO_2 box under fluorescent lights, 2% CO_2, and shaker table set to 175rpm.

2.3.3 Data Collection

2.3.3.1 Store data in a folder named by date (e.g. 120704 for July 4th 2012) and pond name / other experimental identifier.

2.3.3.2 Collect data daily for 4 to 5 days at +/- one hour from 24hour time points of set up. Mix each tube by vortexing and take OD and fluorescence readings. Export data the experimental folder.

2.3.3.3 At least one tube per treatment group should be observed under the microscope (refer to SOP LC 06-001-003 R.0) before performing crop protection activities and as needed.

2.3.4 Reporting

2.3.4.1 Tube 'crashes' (rapid death attributable to biotic agent) are to be immediately reported to pond manager, experiment owner, and Crop Protection team

2.3.4.2 Collected data should be plotted at completion of experiments. Plots should display treatment group averages (±SD) of growth or decline. Graphs and pictures should be compiled into a power point presentation

with a conclusions slide.

3. Required documents

3.1. Input documents

NA

3.2. Output documents

NA

4. Document control

4.1. Revision history

R0 – Initial Release – <Editor name>	<Date>
R1 – <Editor name>	<Date>

4.2. Document approval

<Name> <Approval date>

4.3. Document reviewers

<Name> <Last reviewed date>
<Name> <Last reviewed date>

5. Risk analysis

<Risk name>	<Mitigation plan>	<Owner>	<RPN>