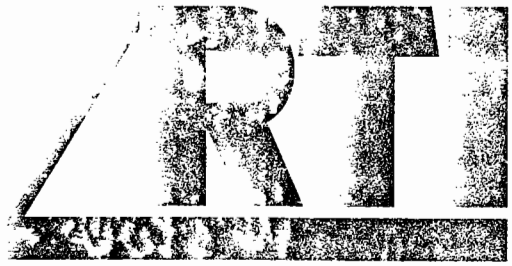

RESEARCH TRIANGLE INSTITUTE

CONFIDENTIAL

Final Report

RTI Project No. 65C-4909-023

Date: June 11, 1993

SIMPLE GREEN® (SG) - End-Use Product Acute Aquatic Toxicity Study with the Cladorean *Daphnia pulex* and Fathead Minnow *Pimephales promelas*

Study Dates: Initiation: April 16, 1992
Completion: June 11, 1993

Prepared
for
Simple Green®
Division of Sunshine Makers, Inc.
15922 Pacific Coast Highway
Huntington Harbour, CA 92649

Prepared by:

Randolph B. Sleet, Ph.D.
Study Director/Head, Comparative and
Environmental Toxicology Laboratory

Approved by:

Frederick J. de Serres, Ph.D.
Research Director
Center for Life Sciences and Toxicology

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GLP COMPLIANCE STATEMENT

This study was performed in compliance with Good Laboratory Practices (GLP) Standards promulgated by the U.S. Environmental Protection Agency, Toxic Substances Control Act (TSCA), Final Rule, *Federal Register* 40, CFR, Part 792, August 17, 1989. The compliance of the procedures followed for test agent formulation with appropriate guidelines was unknown to the Study Director and Research Triangle Institute.

Prepared by:

6/11/93

Randolph B. Sleet, Ph.D. Date
Study Director

Sunshine Makers, Inc.

RESEARCH TRIANGLE INSTITUTE

Chemistry and Life Sciences

Quality Assurance Statement
RTI Project Number: 65C-4909-23

TITLE: Simple Green (SG) - End-Use Product Acute Aquatic Toxicity Study with the Cladorean *Daphnia pulex* and Fathead Minnow *Pimephales promelas*

SPONSOR: Sunshine Makers, Inc.

STUDY CODE NUMBER: Fh92-SG; Protocol Number 428

STUDY DIRECTOR: R. B. Sleet

STUDY DATES: April 16, 1992 through June 11, 1993

The animals used for these studies were obtained form active stock cultures maintained at HydroLogic, Inc., Morrisville, NC. The culturing procedures used at Hydrologic, Inc., follow those described by the US EPA.

Simple Green Batch No. G169 was supplied by the sponsor and the sponsor was responsible for information that appropriately defines strength, purity and composition of Simple Green.

Listed below are the dates of inspections and the dates reports were sent to management.

Critical Phases	Inspection Date	Date Report Signed by Study Director	Date Report Sent to Management
Protocol Review	04-02-92	04-09-92	07-24-92
Lab. Inspection	06-02-92	06-03-92	07-24-92
Test Article Characterization	06-09-92	06-12-92	07-24-92

Post Office Box 12194 Research Triangle Park, North Carolina 27709-2194
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Quality Assurance Statement
Page 2

Critical Phases	Inspection Date	Date Report Signed by Study Director	Date Report Sent to Management
Test Article Character. HPLC	07-09-92	07-10-92	01-07-93
Test Article Characterization GC	07-09-92	07-10-92	01-07-93
Dosing	08-10-92	08-12-92	01-07-93
Water Chemistry	08-10-92	08-12-92	01-07-93
Sacrifice	08-14-92	08-17-92	01-07-93
Dosing-Daphnia RF	08-17-92	08-20-92	01-07-93
Dosing-Fathead	08-24-92	08-25-92	01-07-93
Dose Formulation-Daphnia	08-27-92	09-08-92	01-07-93
Dosing-Fathead	09-14-92	09-14-92	01-07-93
Dosage Analysis	09-14-92	09-18-92	01-07-93
Dose Prep.-Daphnia	09-16-92	09-17-92	01-07-93
Dosage Analysis	09-16-92	09-17-92	01-07-93
Sacrifice-Fathead	09-18-92	09-21-92	01-07-93
Dosage Analysis-GC	11-03-92	11-06-92	01-07-93
Test Article Receipt	11-13-92	11-16-92	01-07-93
Test Article Analysis	11-09-92	11-10-92	01-07-93

The Study Protocol is included in Addendum 1.

The inspections were performed by Mr. David L. Brodish, Mr. Glenn M. Digsby, and Ms. Celia D. Keller. The Final Report audits and Data Audits were performed by Mr. Glenn M. Digsby.

To the best of my knowledge this Final Report accurately describes the study methods and procedures used, and the reported results accurately reflect the raw data.

6-11-93
Date

Glenn M. Digsby
Assistant Manager
Quality Assurance Unit

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SIMPLE GREEN® (SG) - End-Use Product
Acute Aquatic Toxicity Study with the
Cladorean *Daphnia pulex* and Fathead Minnow *Pimephales promelas*

SUMMARY

The overall findings of this study indicate that Simple Green® was practically non-toxic to a freshwater crustacean (*Daphnia pulex*) and fish (fathead minnow, *Pimephales promelas*) at temperatures that are relevant to natural environmental conditions. This interpretation is based on the US EPA classification (US EPA, 1985) which defines water-borne substances as practically non-toxic if they produce 50% lethality in the test population after continuous exposure for 48 or 96 hours at concentrations above 100 ppm.

The acute aquatic toxicity of SG was evaluated at different water temperatures, 25°C (77°F) and 15°C (59°F). For *Daphnia*, the EC_{50} of SG determined at both temperatures was 173 ppm. The LC_{50}s determined for the fathead minnow (*Pimephales promelas*) differed slightly between the two temperatures. At 25°C, the LC_{50} was 87 ppm, and at 15°C, the LC_{50} was 141 ppm. The LC_{50} determined at 25°C is at the high end of the LC_{50} range (> 10 ppm and < 100 ppm) which is classified as slightly toxic by the US EPA (US EPA, 1985). The derived estimates of LC_{50} at 15 °C and 25°C were used to interpolate median lethal concentrations for SG at intermediate water temperatures. Based on the linear relationship defined by the variables, small reductions in temperature would produce proportional increases in the LC_{50} estimates of SG. For example as water temperature decreases below 22°C, the LC_{50} estimates are > 100 ppm, a concentration level that places SG in the practically nontoxic category as defined by the US EPA (US EPA, 1985). Some LC_{50} estimates determined in this way are 110 ppm at 20°C (68°F), 120 ppm at 18°C (64°F) and 130 ppm at 16°C (62°F).

1.0 OBJECTIVE AND OVERVIEW

The purpose of this study was to evaluate the acute aquatic toxicity of SG with freshwater species at different water temperatures, 25°C (77°F) and 15°C (59°F). These temperatures exemplify thermal conditions of a wide range of geographical locations. Daphnids (*Daphnia pulex*) and fathead minnows (*Pimephales promelas*) were used as the test organisms. A range-finding study was performed for each temperature and species to establish doses for the corresponding definitive study. All studies were performed at HydroLogic, Inc., Morrisville, North Carolina, from August 10, 1992 to November 1, 1992. The procedures followed are detailed in the Final Protocol which is attached to this report as Report Addendum 1. Also, included in Report Addendum 1 are Amendments No. 1 and 2 which amend the Final Protocol to account for the modifications that were made for clarification and performance of the approved procedures. The following summarizes the procedures used.