SUMMARY

This study was conducted to determine the potential effects of D1063 on seedling growth and survival of six plant species over 21 days. Twenty-five seedlings per species (5 replicates, each containing 5 plants) were exposed to five concentrations of D1063, a solvent control and a control. Each species was exposed to nominal test concentrations of 1000, 300, 100, 30 and 10 µg A.I./kg, which were selected by the study sponsor. Samples from each exposure level were collected and analyzed one day prior to test initiation, and at test termination.

Plants were grown at ambient carbon dioxide levels under regulated environmental conditions of temperature, light intensity, relative humidity and photoperiod. A nutrient solution containing essential minerals in water was provided daily to the plants by subirrigation. Plants were observed daily for morphological abnormalities and mortality. Shoot length measurements were recorded on test days 0, 1, 3, 5, 7, 14 and 21 (test termination) to determine growth rates. At the conclusion of the test period, measurements of dry shoot and root weights were also recorded. Data generated from plants grown in the presence of D1063 were statistically analyzed to determine if any treatment level caused significant inhibitory or stimulatory effects ($P \leq 0.05$) in comparison to the solvent control data. From the statistical effects observed, a Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) were determined for each species.

Results of chemical analyses established that the treated sand initially contained measured levels of the test material that represented the nominal fortified amounts added (percent recovery range = 92.2 - 110). By test termination, however, the test material concentration had decreased to 28.9% to 69.5% of nominal concentration. Since the rate of test material loss was not established and the maximum exposure occurred at test initiation, the mean measured test concentrations reported in this study were those determined at test initiation. Nominal concentrations of 1000, 300, 100, 30 and 10 µg A.I./kg were measured to be 930, 280, 96, 33 and 11 µg A.I./kg, respectively, one day prior to test initiation.

Supplemental analyses were conducted to confirm the concentration of parent in the treated sand. Sand samples from this study were sent to the Study Sponsor at the initiation and termination (day 21) of the study. The samples were extracted with acetone and analyzed for parent D1063.02R (the radiolabeled form of D1063.01) by Rad-HPLC. At the initiation of the study, 89 - 97% of the radioactivity extracted into acetone and chromatographed as the test substance. At the termination of the study, no parent, D1063.02R, was recovered from two samples. In the remaining seven samples, only 10 - 34% of the radioactivity initially added was recovered as parent.

At test termination, the morphological abnormalities observed (chlorosis, brown leaf tip, necrosis and foliar lesions) in corn, cucumber, ryegrass, tomato and wheat treatment plants did not appear to be related to the concentration of D1063, as compared to the control and solvent control plants. At termination of the soybean test, the presence of foliar lesions on plants exposed to the higher concentrations of the test material did appear to be concentration-related.

Results of Fisher's Exact test demonstrated that the D1063 exposure had no statistically significant effects ($P \leq 0.05$) on survival of the six species tested, except for corn at the lowest treatment level, 11 μg/kg. This effect was not considered to be concentration-related, since survival was not significantly affected at the four higher concentrations. Of the three growth parameters measured (shoot length, shoot weight and root weight) at test termination, no statistically significant reductions ($P \leq 0.05$) were observed for corn or soybean. As compared to the solvent control data, cucumber shoot length was significantly reduced ($P \leq 0.05$) in plants exposed to the two highest concentrations of test material. Shoot length was not reduced for any other species. Shoot weights of cucumber, tomato and wheat plants treated with the highest concentration were significantly reduced as compared to their respective solvent control data. Cucumber, ryegrass and tomato root weights were also significantly reduced at the highest treatment level.

Regression analyses performed on the measured parameters demonstrated that there was a linear effect of concentration ($P \leq 0.05$) on shoot length, shoot weight and root weight of

cucumber and ryegrass. Linear concentration effects were also observed for tomato shoot length and weight. Soybean and wheat shoot weight also demonstrated a significant linear effect of concentration. Only corn exhibited no significant linear concentration effects.

Based on the results of Dunnett's Test, which determined statistically significant reductions in the measured parameters, corn (a monocot) and soybean (a dicot) were determined to be the least sensitive species to the toxicity of D1063, each with a NOEC of 930 µg A.I./kg. Ryegrass (a monocot), tomato and wheat (dicots) were more sensitive to the exposure, each having a NOEC of 280 µg A.I./kg. Cucumber (a dicot) was determined to be the most sensitive species to D1063, with a NOEC of 96 µg A.I./kg. The following table summarizes the results of the seedling growth test with D1063. No stimulatory growth effects of D1063 were observed for any species.

Species	LOEC (µg A.I./kg)[a]	Most Sensitive Parameter(s)	NOEC (µg A.I./kg)[a]
Corn	>930	No effect	930
Cucumber	280	Shoot length	96
Ryegrass	930	Root weight	280
Soybean	>930	No effect	930
Tomato	930	Root weight and shoot weight	280
Wheat	930	Shoot weight	280

[a] Initial measured concentration. The LOEC and NOEC values were determined by the most sensitive parameter, i.e., shoot length, shoot weight, root weight or survival, according to Dunnett's Test or Fisher's Exact Test.

ECOLOGICAL 14

Jop, K.M. *Chronic Toxicity Evaluation of D1063.02R Using Ceriodaphnia dubia Under Static-Renewal Conditions.* Springborn Laboratories, Inc. Report Number 92-01-4080. February 24, 1993.

CHRONIC TOXICITY EVALUATION OF D1063.02R USING *Ceriodaphnia dubia* UNDER STATIC-RENEWAL CONDITIONS

Submitted To:
The Procter and Gamble Company
Ivorydale Technical Center
5299 Spring Grove Avenue
Cincinnati, Ohio 45217

SLI report #92-01-4080

SLI Study #1011-0691-6279-131

STUDY DIRECTOR: Krzysztof M. Jop

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

24 February 1993

FINAL REPORT

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OPERATIONS SECTION

EXECUTIVE SUMMARY

The purpose of this study was to determine the chronic toxicity of D1063.02R to *Ceriodaphnia dubia* at two pH levels. The chronic toxicity tests were conducted at the targeted pH of 7.0 ±0.1 and 8.5 ±0.1.

Biological response of *C. dubia* to D.1063.02R was pH dependent. The chronic toxicity was approximately thirty times lower in slightly basic solution (pH 8.5) than in the neutral solution (pH 7.0). The NOEC at pH 7.0 based on survival and reproduction was 50 µg/L and 6 µg/L, respectively while the NOEC at pH 8.5 based on survival and reproduction was 339 µg/L and 182 µg/L, respectively. The MATC calculated for the short-term chronic test performed at pH 7.0 was 8.5 µg/L and 248 µg/L for the test performed at pH 8.5.

1.0 INTRODUCTION

The objective of this study was to measure the chronic toxicity of D1063.02R to *Ceriodaphnia dubia* under static-renewal test conditions. The chronic toxicity of D1063.02R was defined using the short-term static-renewal toxicity test with *Ceriodaphnia dubia*. Each toxicity test was conducted at constant pH level of 7.0 and 8.5. The definitive toxicity tests were conducted between 3 and 11 December, 1991 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored at SLI.

2.0 MATERIALS AND METHODS

2.1 Protocol

Procedures used in this study followed those described in the SLI protocol entitled "Protocol for 7-day Static Renewal Toxicity Test with *Ceriodaphnia dubia* Following EPA Effluent Guidelines" protocol No. 053091/CD7D.P&G, and five protocol amendments, one issued on 19 June 1991, one issued on 22 October 1991, one issued on 11 November 1991, one issued on

ECOLOGICAL 15

Wuthrich, V. *Influence of FAT 80'023/Q on the Reproduction of Daphnia Magna.* RCC Project Number 262934. September 10, 1990.

Daphnia Repro

R C C

U M W E L T C H E M I E A G

Study Title:

INFLUENCE OF FAT 80'023/Q ON THE REPRODUCTION OF DAPHNIA MAGNA

REPORT:

THIS COPY OF THE REPORT WAS MODIFIED ON MAY 16, 1995 TO COMPLY WITH THE SUBMISSION REQUIREMENTS OF THE EPA

Data Requirement:

OECD No. 202, Part II
EPA 72-2(a) substitute [illegible]

Author:

Mrs. V. Wüthrich

Study Completion Date:

September 10, 1990

Performing Laboratory:

R C C UMWELTCHEMIE AG
P.O. Box
CH - 4452 Itingen/BL
Switzerland

Study Project No. :

RCC PROJECT 262934

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Telephone: 061/98.52.52 / Telex: 966.136 RCC CH
Telefax: 061/98.52.66 + 061/98.52.84

SUMMARY

The influence of FAT 80'023/Q on the reproduction and survival rate of daphnids was investigated in a 21 days semi-static test.

In the study, concentrations in the range 0.01 to 5 mg/l were tested.
The no-effect concentration, taking into account survival rate and reproduction rate, was 0.04 mg/l. The lowest concentration showing toxicity effects on daphnia reproduction was 0.2 mg/l.

The analytical results are summarized as follows:

Nominal Concentration (mg/l)	Time (Days)	% of Nominal
0.01	0	---
0.01	2	---
0.01	19	---
0.01	21	---
0.2	0	76.0
0.2	2	62.8
0.2	19	48.3
0.2	21	77.1
5.0	0	30.1
5.0	2	45.2

ECOLOGICAL 16

Rhodes, J.E., Lotts, J.W., and Hurshman, B.A. *Early Life-Stage Toxicity of Triclosan to the Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions.* ABC Laboratories Project Number 42726. November 27, 1996.

Laboratories

Columbia, Missouri

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SUBMITTED TO

Unilever Research
U.S. 45 River Road
Edgewater, NJ 07020

STUDY TITLE

Early Life-Stage Toxicity of Triclosan to the Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions

DATA REQUIREMENT

U.S. EPA-FIFRA, 40 CFR, Section 158.145
Guideline 72-4

AUTHORS

Jon E. Rhodes, M.S.
Research Scientist/Study Director

Joe W. Lotts, Jr., M.S.
Associate Scientist

Bret A. Hurshman
Scientist

REPORT COMPLETED ON

November 27, 1996

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65202

PROJECT ID

ABC Study #42726

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SUMMARY

A flow-through early life-stage toxicity study was conducted with rainbow trout (*Oncorhynchus mykiss*) exposed to a dilution water control, DMF vehicle blank, and five concentrations of triclosan to determine the maximum acceptable toxicant concentration (MATC) limits. Newly fertilized eggs (<3.5 hours post-fertilization) were placed in exposure chambers to initiate the study, and exposure continued for a total of 96 days. An intermittent flow 2-L proportional diluter system was used to maintain constant test concentrations. Mean measured concentrations of triclosan, determined by high performance liquid chromatography, were 0.0 (control), 0.0 (DMF vehicle blank), 4.26, 8.63, 17.1, 34.1, and 71.3 µg a.i./L. These values ranged from 85 to 89% of the nominal test concentrations which were dilution water control, DMF vehicle blank, 5.0, 10, 20, 40, and 80 µg a.i./L.

Egg hatchability was not affected at any test concentration when compared to the pooled control group. Survival at 35 days was significantly reduced (P=0.00) at 71.3 µg/L (level 5) when compared to the pooled control group. Survival at 61 days post-hatch was significantly reduced (P=0.00) in the 71.3 µg a.i./L (level 5) treatment when compared to the pooled control group.

At 35 days post-hatch, mean standard length was not significantly reduced in any test concentration when compared to the pooled control group. At 61 days post-hatch, mean standard length and dry weight were not significantly reduced in any test concentration when compared to the pooled control group.

Time to 95% hatch was not affected at any test concentration when compared to the pooled control group. Compared to the pooled control group, time to initiation of sustained swim-up behavior was delayed at 71.3 µg a.i./L. Compound-related morphological and behavioral effects were not observed in any test concentration.

Based on the most sensitive endpoint (i.e., survival) evaluated during this early life-stage study, the geometric mean-maximum acceptable toxicant concentration (GM-MATC) for triclosan is estimated to be 49.3 µg a.i./L. The no-observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) are 34.1 and 71.3 µg a.i./L, respectively.

ABC LABS #42726-9