A FLOW-THROUGH LIFE-CYCLE TOXICITY STUDY OF 1-CHLORODODECANE WITH THE CLADOCERAN (*Daphnia magna*)

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 289A-166

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OECD Guideline 211

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STUDY INITIATION DATE: June 13, 2008

STUDY COMPLETION DATE: September 16, 2008

SUBMITTED TO:

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Lonza Inc.

TITLE: A Flow-Through Life-Cycle Toxicity Study of 1-Chlorododecane with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 289A-166

STUDY COMPLETION: September 16, 2008

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the following exceptions:

Periodic analyses of well water for potential contaminants were not conducted in accordance with Good Laboratory Practices, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

The stability of the test substance under the conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

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Senior Biologist

Date

SPONSOR APPROVAL:

Joseph R. Robinson — 9/16/08

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Date

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Protocol	May 13, 2008	May 13, 2008	May 20, 2008
Test Substance Preparation	June 13, 2008	June 16, 2008	June 16, 2008
Observations	August 11, 2008	August 11, 2008	August 15, 2008
Matrix Fortification	August 14, 2008	August 14, 2008	August 14, 2008
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Biological Data and Draft Report	September 6-8, 2008	September 8, 2008	September 9, 2008
Final Report	September 16, 2008	September 16, 2008	September 16, 2008

All inspections were study-based.

Linda R. Michell

Linda R. Michell
Director of Regulatory and Ecotox Operations

16 SEPTEMBER 2008
Date

REPORT APPROVAL

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WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 289A-166

STUDY DIRECTOR:

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SUMMARY

SPONSOR: Lonza Inc.

TITLE: A Flow-Through Life-Cycle Toxicity Study of 1-Chlorododecane with the Cladoceran (*Daphnia magna*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 289A-166

TEST DATES:	Study Initiation:	June 13, 2008
	Experimental Start (OECD):	July 18, 2008
	Experimental Start (EPA):	July 24, 2008
	Biological Termination:	August 14, 2008
	Experimental Termination:	August 18, 2008

LENGTH OF EXPOSURE: 21 Days

TEST ORGANISM: Cladoceran (*Daphnia magna*)

SOURCE OF TEST ORGANISMS: Wildlife International, Ltd.
Easton, Maryland 21601

AGE OF TEST ORGANISMS: Neonates (< 24 hours old)

TEST CONCENTRATIONS:

Nominal (µg a.i./L)	Mean Measured (µg a.i./L)
Negative Control	< LOQ
Solvent Control	< LOQ
6.3	1.5
13	2.7
25	5.7
50	7.2
100	17

Efforts to determine the cause of the low measured concentrations were unsuccessful and it was assumed that the analytical recoveries were affected by the very low water solubility of 1-Chlorododecane (Wildlife International, Ltd. Project No.: 289C-203). It was concluded that conduct of the study at the specified concentrations adequately represented the potential toxicity of 1-Chlorododecane to *Daphnia*. Therefore, the study was conducted as described and the results of the study were based on the mean measured concentrations.

RESULTS: The most sensitive end-point was growth.

No-Observed-Effect-Concentration:	7.2 µg a.i./L
Lowest-Observed-Effect-Concentration:	17 µg a.i./L
Maximum Acceptable Toxicant Concentration:	11 µg a.i./L
21-Day EC50 (Adult Mortality/Immobility):	>17 µg a.i./L
21-Day EC50 (Reproduction):	>17 µg a.i./L

INTRODUCTION

This study was conducted by Wildlife International, Ltd. for Lonza Inc. at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The in-life phase of the test was conducted from July 24 to August 14, 2008, with dry weight measurements completed on August 18, 2008. Raw data generated by Wildlife International, Ltd. and the original final report are filed under Project Number 289A-166 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the effects of 1-Chlorododecane on the survival, growth and reproduction of the cladoceran, *Daphnia magna*, under flow-through test conditions, for a period of 21days exposure.

EXPERIMENTAL DESIGN

Daphnids were exposed to a geometric series of five test concentrations, a negative control (dilution water) and a solvent control (dimethyl formamide) for 21 days. Nominal test concentrations selected for the test were 6.3, 13, 25, 50 and 100 µg active ingredient (a.i.)/L. The concentrations were selected in consultation with the Sponsor, and were based upon the results of exploratory range finding toxicity data. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination.

Delivery of the test solutions to the test chambers was initiated six days prior to test initiation in order to achieve equilibrium of the test substance. Two replicate test chambers were tested for each treatment and control. Each replicate contained two compartments with five daphnids, resulting in a total of 20 *Daphnia magna* in each treatment and control group. At test initiation, neonate daphnids were impartially assigned to exposure chambers. First-generation daphnids were observed daily during the test for mortality, the onset of reproduction, and clinical signs of toxicity. Following the onset of reproduction, the numbers of second-generation daphnids were counted three times per week (i.e., Monday, Wednesday and Friday). Body lengths and dry weights of the surviving first-generation daphnids were measured at the end of the exposure period. Observations of the effects of 1-Chlorododecane on survival, reproduction and growth were used to determine the no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), and the maximum

acceptable toxicant concentration (MATC). EC50 values were determined based on reproduction and on first-generation mortality/immobility at test termination.

MATERIALS AND METHODS

The study was conducted according to the protocol, "A Flow-Through Life-Cycle Toxicity Study of 1-Chlorododecane with the Cladoceran (*Daphnia magna*)" (Appendix 1). The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines, OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test* (1); OECD Guideline 211: *Daphnia magna Reproduction Test* (2); and ASTM Standard E 1193-97: *Standard Guide for Conducting Daphnia magna Life-Cycle Toxicity Tests* (3).

Test Substance

The test substance used to prepare the test solutions, analytical matrix fortification samples, and calibration standards for the study was received from Lonza Inc. on May 24, 2007. It was assigned Wildlife International, Ltd. identification number 8134 upon receipt and was stored under ambient conditions. The test substance, a liquid, was identified as: Barchlor 12s (1-Chlorododecane); CAS No. 112-52-7; Batch number N6227945. The test substance had a reported purity of 97.42% and an expiration date of June 29, 2009. A copy of the test substance Certificate of Analysis is presented in Appendix 2.

Test Organism

The cladoceran, *Daphnia magna*, was selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates and were selected for use in the test based upon past history of use and ease of culturing in the laboratory. Daphnid neonates used in the test were less than 24 hours old and were obtained from cultures identified by species and maintained by Wildlife International, Ltd., Easton, Maryland.

Adult daphnids were cultured in water from the same source and at approximately the same temperature as used during the test. During the 14-day period preceding the test, water temperatures ranged from 20.3 to 20.9ºC, measured with a hand-held liquid-in-glass thermometer. The pH of the water ranged from 8.2 to 8.6, measured with a Fisher Scientific Accumet Model 915 pH meter. Dissolved oxygen concentrations ranged from 7.6 to 9.2 mg/L (≥84% of saturation), measured with a Yellow Springs Instruments Model 85 dissolved oxygen meter.

The adult daphnids in the cultures were at least 22 days old prior to collection of the juveniles for testing. The adults showed no signs of disease or stress during the culture period, and no ephippia were produced in the cultures. Neonate daphnids were obtained for testing from seven individual adult daphnids that had produced at least one prior brood. At test initiation, the juvenile daphnids were collected from the cultures and indiscriminately transferred one and two at a time to transfer chambers (e.g., 10 mL glass beakers) until each chamber contained five neonates. Each transfer chamber was indiscriminately assigned to a test compartment, and the neonates were transferred to the test compartments to initiate the test. All transfers were made below the water surface using wide-bore pipettes.

During culture and testing, daphnids were fed a mixture of yeast, cereal grass, and trout chow, as well as a suspension of the freshwater green alga, *Pseudokirchneriella subcapitata*. Daphnids were fed daily during culturing, at least two times per day through Day 7 of the test and then were fed four times per day until the last day of the test. There were no known levels of contaminants reasonably expected to be present in the diet that were considered to interfere with the purpose or conduct of the test.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, pH and total organic carbon content of the well water during the four-week period immediately preceding the test are presented in Appendix 3.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use, the water again was filtered (0.45 µm) and was passed through an ultraviolet (UV) sterilizer to remove microorganisms and particles. The results of periodic analyses performed to measure the concentrations of selected organic and inorganic constituents in the well water are presented in Appendix 4.

Test Apparatus

A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control and a negative control (dilution water). Syringe pumps (Harvard Apparatus, Massachusetts) were used to deliver five test substance stock solutions and dimethylformamide (DMF) for the solvent control into mixing chambers indiscriminately assigned to each treatment and the solvent control. The syringe pumps were calibrated prior to the test. The stock solutions were diluted with well water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters, which were calibrated prior to test initiation and at approximately weekly intervals thereafter. The flow of test water from each mixing chamber was split and allowed to flow into two replicate test chambers. The proportion of the test water that was split into each replicate was checked prior to the test and at approximately weekly intervals thereafter to ensure that flow rates varied by no more than ±10% of the mean for the two replicates. The diluter flow rate was adjusted to provide approximately five volume additions of test water in each test chamber per day. Delivery of the test solutions to the test chambers was initiated six days prior to test initiation in order to achieve equilibrium of the test substance. The general operation of the diluter was checked visually at least two times per day at the beginning of the test and during the test, and once at the end of the test.

Test chambers were indiscriminately positioned in a temperature-controlled water bath to maintain the target temperature throughout the test period. The water bath was enclosed in a plexiglass ventilation hood in order to minimize potential cross-contamination between test systems. The daphnids were held in two test compartments suspended in each of two test chambers, for a total of four test compartments per control or treatment group. The test chambers were 25-L, Teflon®-lined, stainless steel aquaria filled with approximately 22 L of test solution. Test compartments were 300 mL glass beakers, approximately 6.5 cm in diameter and 12 cm in height. Nylon mesh screens covered two holes on opposite sides of each test compartment to permit test solution to flow in and out of the compartment. The depth of the test water in a representative test compartment was approximately 8 cm, while the depth of water in a representative test chamber was approximately 29 cm. All test chambers were labeled with the project number, test concentration and chamber replicate; test compartments within each test chamber were labeled with test concentration and compartment replicate.

Preparation of Test Concentrations

Stock solutions were prepared three times during the test. The stock and test concentrations were prepared on the basis of active ingredient (a.i.) of the test substance. A primary stock solution

was prepared in DMF at a nominal concentration of 1000 µg a.i./mL. Proportional dilutions of the primary stock were made in DMF to prepare additional stock solutions at nominal concentrations of 63, 130, 250 and 500 µg a.i./mL. The stock solutions were mixed by inverting at least 20 times, and were clear and colorless in appearance. The stock solutions were delivered to the diluter mixing chambers (at a target rate of 15.5 µL/minute) where they were mixed with dilution water (at a nominal rate of 155 mL/minute) to achieve the desired test concentrations of 6.3, 13, 25, 50 and 100 µg a.i./L. The solvent control was prepared by injecting DMF into the mixing chamber assigned to the solvent control. The concentration of DMF in the solvent control and all treatment groups was 0.1 mL/L. The test solutions appeared clear and colorless in the diluter mixing chambers and in the test chambers at test initiation and termination.

Analytical Sampling

Water samples were collected from one test chamber of each treatment and control group three days prior to the start of the test to confirm the operation of the diluter. Water samples also were collected from alternating replicate test chambers at the beginning of the test, at weekly intervals during the test and at the end of the test to determine concentrations of the test substance. All samples were collected at mid-depth from each test chamber, placed in a separator funnel and processed immediately for analysis.

Analytical Method

The method used for the analysis of 1-Chlorododecane in freshwater samples was based upon methodology developed by Wildlife International, Ltd. The analytical method consisted of extracting the freshwater samples with ethyl acetate and analyzing by gas chromatography with mass selective detection.

Concentrations of 1-Chlorododecane in the samples were determined using a Hewlett-Packard Model 5890 Gas Chromatograph (GC) with a Hewlett-Packard Model 5971A Mass Selective Detector operated in the Selective Ion Monitoring (SIM) mode. Chromatographic separations were achieved using a ZB-5 column (30 m x 0.25 mm ID, 0.25 µm). The method flowchart is provided in Appendix 5.1 and the instrument parameters are summarized in Appendix 5.2.

Calibration standards of 1-Chlorododecane, ranging in concentration from 4.50 to 60.00 µg a.i./L for the definitive test, were prepared using stock solutions of 1-Chlorododecane in acetone (Appendix 5.3). Calibration standards were analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of 1-Chlorododecane in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equations. An example of the calculations for a representative sample is included in Appendix 5.4.

The method limit of quantitation (LOQ) for the definitive analyses was defined as 2.25 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (4.50 µg a.i./L) and the dilution factor of the matrix blank samples (0.500). Four matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses (Appendix 5.5).

Samples of freshwater were fortified at 6.00, 25.0 and 100 µg a.i./L using stock solutions of 1-Chlorododecane in acetone (Appendix 5.3), and were analyzed concurrently with each test sample set. The measured concentrations for the matrix fortification samples ranged from 75.1 to 114% of nominal concentrations (Appendix 5.5).

A representative calibration curve is presented in Appendix 5.6. Representative chromatograms of low- and high-level calibration standards are presented in Appendices 5.7 and 5.8, respectively. Representative chromatograms of a matrix blank sample and a matrix fortification sample are presented in Appendices 5.9 and 5.10, respectively. A representative chromatogram of a test sample is presented in Appendix 5.11.

Environmental Conditions

Fluorescent light bulbs that emit wavelengths similar to natural sunlight (Colortone® 50) were used for illumination of the cultures and test chambers. A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light

intensity at test initiation was 177 lux over one representative test chamber, and was measured using a SPER Scientific Model 840006C light meter.

The target test temperature was 20 ± 1ºC. Temperature was measured in each test chamber at test initiation and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control test chamber using a Fulscope ER/C Recorder, which was verified in the same control chamber prior to test initiation and approximately weekly during the test using a liquid-in-glass thermometer.

Dissolved oxygen was measured in alternating replicate test chambers of each treatment and control group at the beginning and end of the test, and approximately three times per week during the test. Measurements of pH were made in alternating replicate test chambers of each treatment and control group at test initiation and at weekly intervals thereafter. Dissolved oxygen was measured using a Thermo Orion 850Aplus dissolved oxygen meter, and measurements of pH were made using a Thermo Orion 525Aplus pH meter.

Hardness, alkalinity and specific conductance were measured in alternating replicates of the negative control (dilution water) and the highest test concentration at test initiation and at weekly intervals thereafter. Total organic carbon (TOC) was measured in the dilution water at test initiation and termination. Hardness and alkalinity were measured by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (4). Specific conductance was measured using an Acorn Series CON 6 Conductivity Meter. Total organic carbon was measured using a Shimadzu Model TOC-VCSH Total Organic Carbon Analyzer.

Biological Observations and Measurements

Observations of each first-generation daphnid were made daily during the test. At these times, the numbers of dead and immobile daphnids were recorded along with any clinical signs of toxicity (e.g., inability to maintain position in the water column, uncoordinated swimming or cessation of feeding). Immobility was defined as a lack of movement, except for minor spontaneous random movement of the appendages. The presence of eggs in the brood pouch, aborted eggs, males or ephippia also were recorded daily. With the onset of reproduction, neonates produced by the first-generation daphnids were counted and then discarded every Monday, Wednesday and Friday during

the test. Onset of reproduction is defined by the first observation of neonates in a test compartment and was used to calculate the time to first brood, calculated as the number of days after test initiation until the offspring were first observed for each compartment. The body length and the dry weight of each surviving first-generation daphnid were measured at the end of the test.

Statistical Analyses

Test endpoints analyzed statistically for first-generation daphnids were survival, reproduction (the numbers of live young produced per reproductive day), and growth (length and dry weight). A reproductive day was defined as each day a first-generation daphnid was alive following the onset of reproduction for any test control or treatment group (e.g. 5 first-generation daphnids in a test compartment for one day after onset of reproduction represent 5 reproductive days). Negative control and solvent control data for each parameter were compared using an appropriate statistical test. No significant differences between the control groups were found for any parameter tested ($p > 0.05$). Therefore, the control data were pooled for comparison with the treatment groups.

Survival data was considered to be discrete-variable data, while reproduction and growth data were considered continuous-variable data. Discrete-variable data were analyzed using Chi-square and Fisher's Exact tests to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the pooled control. All continuous-variable data were evaluated for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test ($p = 0.01$). Since the data for all parameters passed the assumptions of normality and homogeneity, an analysis of variance (ANOVA) was used to determine whether or not statistically significant differences existed among the experimental groups ($p = 0.05$). Those treatments that were significantly different from the control means were identified using Dunnett's t-test ($p \leq 0.05$). All statistical tests were performed using a personal computer with SAS (5) software.

The results of the statistical analyses were used to aid in the determination of the NOEC, LOEC and MATC. However, scientific judgement was used to determine if statistical differences were biologically meaningful, and if the data followed a concentration-dependent response. The NOEC was defined as the highest test concentration that produced no significant treatment-related effects on survival, reproduction or growth. The LOEC was defined as the lowest test concentration that produced a significant treatment-related effect on survival, reproduction or growth. The MATC was calculated as the geometric mean of the NOEC and LOEC. EC50 values were determined based on cumulative

reproduction and mortality/immobility observed in the first-generation daphnids at the end of the test (6, 7).

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Nominal concentrations selected for use in the study were 6.3, 13, 25, 50 and 100 µg a.i./L. Results of the pretest diluter verification sample analyses are presented in Table 1, and the results of the weekly sample analyses during the test are presented in Table 2. The measured concentrations of the pretest samples ranged from approximately 14 to 39% of nominal concentrations. The measured concentrations of samples collected from each test concentration on Days 0, 7, 14 and 21 ranged from 6 to 40% of nominal concentrations. The measured concentrations of test solution samples collected weekly during the test were averaged for each treatment group, yielding the mean measured test concentrations of 1.5, 2.7, 5.7, 7.2 and 17 µg a.i./L, which represented 24, 21, 23, 14 and 17% of nominal concentrations, respectively. When individual analytical concentrations were less than the LOQ, a value of ½ LOQ (1.125 µg a.i./L) was used in the calculation of the mean measured concentration. Efforts to determine the cause of the low measured concentrations were unsuccessful and it was assumed that the analytical recoveries were affected by the very low water solubility of 1-Chlorododecane (Wildlife International, Ltd. Project No.: 289C-203). It was concluded that conduct of the study at the specified concentrations adequately represented the potential toxicity of 1-Chlorododecane to *Daphnia*. Therefore, the study was conducted as described and the results of the study were based on the mean measured concentrations.

Water Chemistry Measurements

Water temperatures measured weekly in the test chambers ranged from 20.0 to 20.3°C (Table 3). Temperatures measured continuously in one negative control chamber were within the 20 ± 1°C range specified in the protocol. Dissolved oxygen concentrations in the test chambers remained ≥68% of saturation (6.1 mg/L) throughout the test period (Table 4). Measurements of water pH in the test chambers ranged from 8.1 to 8.3 throughout the test period (Table 5). Weekly measurements of specific conductance, hardness and alkalinity in the negative control water and in the highest test concentration (17 µg a.i./L) were consistent over the test period (Table 6). TOC in the dilution water at test initiation and termination was <1 mg C/L (Table 6).

Survival and Clinical Observations

A summary of the observations of cumulative mortality, immobility and sublethal signs of toxicity in the first-generation daphnids is shown in Table 7. Daily observations are presented in Appendix 6. After 21 days of exposure, survival in the negative and solvent control groups was 95 and 85%, respectively. There was no significant difference in survival between the negative and solvent control groups. Therefore, the control data were pooled for comparison with the treatment groups. Survival in the 1.5, 2.7, 5.7, 7.2 and 17 µg a.i./L treatment groups was 100, 100, 95, 95 and 95%, respectively, at test termination. There were no statistically significant ($p \leq 0.05$) reductions in survival at any of the concentrations tested in comparison to the pooled controls (Fisher's Exact test). Consequently, the NOEC for survival was 17 µg a.i./L and the LOEC was >17 µg a.i./L. Since less than 50% mortality/immobility was observed among first-generation daphnids in any treatment group by test termination, an EC50 value could not be calculated. Surviving daphnids in the control groups and in the 1.5, 2.7, 5.7, 7.2 and 17 µg a.i./L treatment groups appeared normal at test termination.

Reproduction

A summary of neonate production by first-generation daphnids is presented in Table 8. A complete listing of the number of neonates counted in each test compartment on each observation day is provided in Appendix 7. With the exception of one replicate in the 7.2 µg a.i./L treatment group and one replicate in the 17 µg a.i./L treatment group, the first day of brood production in the negative and solvent control replicates and in all 1-Chlorododecane treatment replicates was Day 7 or 8 of the test, indicating that there was no apparent delay in the onset of production at any 1-Chlorododecane concentration tested. The first day of brood production for Replicate C of the 7.2 µg a.i./L treatment group and Replicate A of the 17 µg a.i./L treatment group was Day 11. With the exception of two immobile neonates in the 2.7 µg a.i./L treatment at test termination, no immobile neonates were noted in the control or treatment groups during the test (Table 8). No aborted or shed eggs were observed during the test. No males or ephippia were produced during the test.

Adult daphnids in the negative and solvent control groups produced an average of 8.2 and 9.1 live young per reproductive day, respectively. There was no significant difference in reproduction between the negative and solvent control groups. Therefore, the control data were pooled for comparison with the treatment groups. Daphnids in the 1.5, 2.7, 5.7, 7.2 and 17 µg a.i./L treatment groups produced an average of 9.4, 9.2, 9.1, 9.4 and 7.7 live young per reproductive day, respectively.

When compared to the pooled control group, Dunnett's test indicated that there were no statistically significant ($p > 0.05$) differences in reproduction at any of the concentrations tested. Consequently, the NOEC for reproduction was 17 µg a.i./L, the highest concentration tested, and the LOEC was >17 µg a.i./L. The 21-day EC50 value for reproduction was >17 µg a.i./L.

Growth

A summary of the mean lengths and dry weights of surviving first-generation daphnids are presented in Table 9. Individual growth measurements are provided in Appendix 8. Daphnids in the negative control group averaged 5.6 mm in length and 1.08 mg in dry weight, while daphnids in the solvent control group averaged 5.5 mm in length and 0.99 mg in dry weight. There were no significant differences in the growth parameters between the negative and solvent control groups. Therefore, the control data were pooled for comparison with the treatment groups. Daphnids in the 1.5, 2.7, 5.7, 7.2 and 17 µg a.i./L treatment groups had mean lengths of 5.5, 5.5, 5.6, 5.7 and 5.2 mm, respectively, and mean dry weights of 1.07, 0.95, 1.08, 1.01 and 0.94 mg, respectively. When compared to the pooled control group, Dunnett's test indicated there was a statistically significant decrease in mean length ($p \leq 0.01$) and dry weight ($p \leq 0.05$) in the 17 µg a.i./L treatment group, and in dry weight ($p \leq 0.05$) in the 2.7 µg a.i./L treatment group. The decrease in dry weight in the 2.7 µg a.i./L treatment group was slight, not associated with a decrease in mean length and was not dose-responsive. Therefore the decrease in dry weight for the 2.7 µg a.i./L treatment group was not considered to be treatment-related. The decrease in mean length in the 17 µg a.i./L was both strongly significant ($p \leq 0.01$) and associated with a significant decrease in dry weight. Therefore, impacts on growth at the 17 µg a.i./L test concentration were considered to be treatment-related. Consequently, the NOEC for growth (based on length and dry weight) was 7.2 µg a.i./L, and the LOEC was 17 µg a.i./L.

CONCLUSIONS

Daphnia magna exposed to 1-Chlorododecane at mean measured concentrations of 1.5 to 17 µg a.i./L for 21 days showed no significant treatment-related reductions in survival or reproduction at concentrations of 17 µg a.i./L and below, or in growth at concentrations of 7.2 µg a.i./L and below. Treatment-related reductions in growth (length and dry weight) were apparent at the 17 µg a.i./L test concentration, the highest concentration tested. Consequently, the NOEC for this study, based on the most sensitive endpoint tested (growth) was 7.2 µg a.i./L and the LOEC was 17 µg a.i./L. The

MATC was calculated to be 11 µg a.i./L. The 21-day EC50 value for adult mortality/immobility and reproduction was >17 µg a.i./L.

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Table 1

Measured Concentrations of 1-Chlorododecane in Pretest Diluter Verification Samples

Nominal Concentration (µg a.i./L)	Sample Number (289A-166-)	Sampling Time (Day)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]
Negative Control 0.0	PT-1	-3	< LOQ	--
Solvent Control 0.0	PT-2	-3	< LOQ	--
6.3	PT-3	-3	2.48	39.3
13	PT-4	-3	4.51	34.7
25	PT-5	-3	9.73	38.9
50	PT-6	-3	17.5	35.1
100	PT-7	-3	13.6	13.6

[1] The limit of quantitation (LOQ) was 3.00 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (6.00 µg a.i./L) and the dilution factor of the matrix blank samples (0.500).
[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 2

Measured Concentrations of 1-Chlorododecane in Test Solution Samples

Nominal Test Concentration (µg a.i./L)	Sample Number (289A-166-)	Sampling Time (Hours)	Measured Concentration (µg a.i./L)[1]	Percent of Nominal[2]	Mean Measured Concentration[3] (µg a.i./L)	Mean Percent of Nominal
Negative Control (0.0)	1	0	< LOQ	--	--	--
	8	7	< LOQ	--		
	15	14	< LOQ	--		
	22	21	< LOQ	--		
Solvent Control (0.0)	2	0	< LOQ	--	--	--
	9	7	< LOQ	--		
	16	14	< LOQ	--		
	23	21	< LOQ	--		
6.3	3	0	2.51	39.8	1.5	24
	10	7	< LOQ	--		
	17	14	< LOQ	--		
	24	21	< LOQ	--		
13	4	0	4.14	31.8	2.7	21
	11	7	< LOQ	--		
	18	14	2.93	22.6		
	25	21	2.67	20.6		
25	5	0	6.87	27.5	5.7	23
	12	7	4.07	16.3		
	19	14	5.46	21.8		
	26	21	6.53	26.1		
50	6	0	11.4	22.8	7.2	14
	13	7	8.07	16.1		
	20	14	3.72	7.44		
	27	21	5.44	10.9		
100	7	0	24.0	24.0	17	17
	14	7	23.6	23.6		
	21	14	6.32	6.32		
	28	21	15.4	15.4		

[1] The limit of quantitation (LOQ) was 2.25 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (4.50 µg a.i./L) and the dilution factor of the matrix blank samples (0.500).
[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.
[3] Mean measured concentrations were calculated using a value of ½ LOQ (1.125 µg a.i./L) when a reported value was less than the LOQ.

Table 3

Temperature of Water in the Test Chambers

Mean Measured Concentration (µg a.i./L)	Test Chamber Replicate	Temperature (°C)			
		Day 0	Day 7	Day 14	Day 21
Negative Control	A[1]	20.2	20.2	20.0	20.3
	B	20.2	20.2	20.0	20.2
Solvent Control	A	20.2	20.2	20.0	20.2
	B	20.2	20.2	20.0	20.1
1.5	A	20.2	20.2	20.0	20.1
	B	20.2	20.2	20.0	20.1
2.7	A	20.2	20.2	20.0	20.1
	B	20.2	20.2	20.0	20.1
5.7	A	20.1	20.1	20.0	20.0
	B	20.2	20.2	20.0	20.0
7.2	A	20.1	20.2	20.0	20.1
	B	20.2	20.2	20.0	20.1
17	A	20.2	20.2	20.0	20.0
	B	20.2	20.2	20.0	20.1

[1] Temperature measured continuously in Control Replicate A during the test was approximately 20.0°C, measured to the nearest 0.5°C.

Table 4

Dissolved Oxygen Concentration of Water in the Test Chambers

Mean Measured Test Concentration (µg a.i./L)	Replicate	Dissolved Oxygen Concentration (mg/L)[1]									
		Day 0	Day 4	Day 6	Day 8	Day 11	Day 13	Day 15	Day 18	Day 20	Day 21
Negative Control	A	8.6	--	8.7	--	8.5	--	8.6	--	8.5	--
	B	--	8.5	--	8.5	--	8.6	--	8.6	--	8.6
Solvent Control	A	8.5	--	7.3	--	7.4	--	8.6	--	6.5	--
	B	--	7.0	--	8.5	--	6.2	--	6.8	--	6.1
1.5	A	8.3	--	6.7	--	7.4	--	8.8	--	6.5	--
	B	--	6.5	--	8.0	--	6.9	--	7.0	--	6.5
2.7	A	8.6	--	6.6	--	7.4	--	6.4	--	6.4	--
	B	--	6.4	--	8.0	--	7.1	--	6.9	--	6.5
5.7	A	8.5	--	6.7	--	6.9	--	6.7	--	6.2	--
	B	--	7.0	--	7.9	--	7.2	--	7.0	--	6.6
7.2	A	8.6	--	6.4	--	6.9	--	6.3	--	6.4	--
	B	--	7.3	--	8.1	--	6.7	--	6.9	--	6.5
17	A	8.5	--	6.3	--	6.9	--	6.4	--	6.2	--
	B	--	6.6	--	8.1	--	6.7	--	6.6	--	6.5

[1] A dissolved oxygen concentration of 5.4 mg/L represents 60% saturation at 20ºC in freshwater.

Table 5

pH of Water in the Test Chambers

Mean Measured Concentration (µg a.i./L)	Replicate	pH			
		Day 0	Day 7	Day 14	Day 21
Negative Control	A	8.2	--	8.2	--
	B	--	8.2	--	8.2
Solvent Control	A	8.2	--	8.1	--
	B	--	8.3	--	8.0
1.5	A	8.2	--	8.1	--
	B	--	8.2	--	8.1
2.7	A	8.2	--	8.2	--
	B	--	8.3	--	8.1
5.7	A	8.2	--	8.1	--
	B	--	8.2	--	8.1
7.2	A	8.2	--	8.1	--
	B	--	8.2	--	8.1
17	A	8.2	--	8.1	--
	B	--	8.2	--	8.1

Table 6

Specific Conductance, Hardness and Alkalinity in the Negative Control and the Highest Concentration Treatment Group

Negative Control

Parameter	Test Day			
	0[1]	7	14	21[1]
Specific Conductance (µS/cm)	352	359	358	359
Hardness (mg/L as $CaCO_3$)	134	138	136	140
Alkalinity (mg/L as $CaCO_3$)	178	181	182	178
Replicate	A	B	A	B

[1] TOC measured in the dilution water at test initiation and termination was <1 mg C/L.

17 µg a.i./L Treatment Group

Parameter	Test Day			
	0	7	14	21
Specific Conductance (µS/cm)	366	362	370	373
Hardness (mg/L as $CaCO_3$)	136	140	136	128
Alkalinity (mg/L as $CaCO_3$)	179	180	184	186
Replicate	A	B	A	B

Table 7

Summary of Cumulative Mortality, Immobility and Clinical Observations of First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Number Daphnids Exposed	Day 7			Day 14			Day 21			Percent Survival [2,3]
		% Dead	% Immobile	Observations [1]	% Dead	% Immobile	Observations [1]	% Dead	% Immobile	Observations [1]	
Negative Control	20	0	0	20 AN	0	0	20 AN	5	0	19 AN	95
Solvent Control	20	0	0	20 AN	5	0	19 AN	15	0	17 AN	85
1.5	20	0	0	20 AN	0	0	20 AN	0	0	20 AN	100
2.7	20	0	0	20 AN	0	0	20 AN	0	0	20 AN	100
5.7	20	0	0	20 AN	5	0	19 AN	5	0	19 AN	95
7.2	20	0	0	20 AN	5	0	19 AN	5	0	19 AN	95
17	20	0	0	20 AN	5	0	19 AN	5	0	19 AN	95

[1] Observations: AN = appear normal.
[2] 21-Day EC50 was estimated to be >17 µg a.i./L.
[3] There were no statistically significant differences ($p > 0.05$) from the pooled controls (90% pooled survival) using Fisher's Exact test.

Table 8

Summary of Reproduction of First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	First Day of Reproduction	Number of Live Young Produced Per Reproductive Day	Mean Number of Live Young Produced Per Reproductive Day (± SD)[1,2]
Negative Control	A	7	8.7	8.2 ± 0.42
	B	8	8.1	
	C	8	7.7	
	D	7	8.3	
Solvent Control	A	7	8.0	9.1 ± 0.73
	B	7	9.4	
	C	7	9.6	
	D	7	9.3	
Pooled Controls	--	--		8.6 ± 0.72
1.5	A	7	9.0	9.4 ± 0.90
	B	7	8.4	
	C	7	9.6	
	D	8	10.5	
2.7	A	8	8.8	9.2 ± 0.67
	B	8	10.2[3]	
	C	8	8.9	
	D	8	8.9	
5.7	A	8	8.6	9.1 ± 0.39
	B	8	9.5	
	C	8	8.9	
	D	8	9.2	
7.2	A	8	9.6	9.4 ± 0.72
	B	8	9.8	
	C	11	8.3	
	D	8	9.8	
17	A	11	5.7	7.7 ± 1.46
	B	8	8.0	
	C	8	7.9	
	D	8	9.2	

[1] 21-Day EC50 was >17 µg a.i./L.
[2] There were no statistically significant differences ($p > 0.05$) from the pooled controls using the Dunnett's test
[3] On Day 21, two immobile neonates were present in the test compartment.

Table 9

Summary of Length and Dry Weight of Surviving First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	Number Surviving	Compartment Mean Length (mm)	Treatment Mean Length (mm) (±SD)	Compartment Mean Dry Weight (mg)	Treatment Mean Dry Weight (mg) (±SD)
Negative Control	A	5	5.5	5.6 ± 0.05	1.07	1.08 ± 0.074
	B	4	5.6		1.07	
	C	5	5.6		0.99	
	D	5	5.6		1.17	
Solvent Control	A	5	5.3	5.5 ± 0.13	0.91	0.99 ± 0.057
	B	4	5.6		1.02	
	C	4	5.5		1.00	
	D	4	5.5		1.04	
Pooled Controls	--	--		5.5 ± 0.10	--	1.03 ± 0.075
1.5	A	5	5.4	5.5 ± 0.13	1.00	1.07 ± 0.051
	B	5	5.5		1.12	
	C	5	5.5		1.09	
	D	5	5.7		1.08	
2.7	A	5	5.2	5.5 ± 0.22	0.91	0.95 ± 0.035*
	B	5	5.7		0.97	
	C	5	5.6		0.92	
	D	5	5.5		0.98	
5.7	A	5	5.7	5.6 ± 0.10	1.09	1.08 ± 0.047
	B	5	5.6		1.01	
	C	4	5.7		1.12	
	D	5	5.5		1.08	
7.2	A	5	5.6	5.7 ± 0.06	1.06	1.01 ± 0.042
	B	5	5.6		1.03	
	C	4	5.7		0.98	
	D	5	5.7		0.97	
17	A	5	4.9	5.2 ± 0.19**	0.87	0.94 ± 0.054*
	B	5	5.2		0.92	
	C	4	5.3		1.00	
	D	5	5.3		0.95	

* Statistically significant difference ($p \leq 0.05$) from the pooled controls using the Dunnett's test.
** Statistically significant difference ($p \leq 0.01$) from the pooled controls using the Dunnett's test.

Appendix 1

Protocol and Protocol Amendments

P R O T O C O L

A FLOW-THROUGH LIFE-CYCLE TOXICITY STUDY OF
1-CHLORODODECANE WITH THE CLADOCERAN (*Daphnia magna*)

U.S. EPA OPPTS Number 850.1300

ASTM E 1193-97

and

OECD Guideline 211

Wildlife International, Ltd. Project Number: 289A-166

Submitted to

Lonza Inc.
90 Boroline Road
Allendale, NJ 07401

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

February 15, 2008

Wildlife International, Ltd.

A FLOW-THROUGH LIFE-CYCLE TOXICITY STUDY OF
1-CHLORODODECANE WITH THE CLADOCERAN (*Daphnia magna*)

SPONSOR:	Lonza Inc. 90 Boroline Road Allendale, NJ 07401
SPONSOR'S REPRESENTATIVE:	John P. Van Miller, Ph.D., DABT Toxicology/Regulatory Services, Inc. 2365 Hunters Way Charlottesville, VA 22911
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Sean P. Gallagher, Senior Biologist Wildlife International, Ltd.
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology/Terrestrial Plants & Insects

FOR LABORATORY USE ONLY

Proposed Dates:
Experimental Start Date: 6/19/08 Experimental Termination Date: 7/10/08
Project No.: 289A-166
Test Concentrations: Neg. control, Sol. control, 6.3, 13, 25, 50 and 100 ug a.i./L
Test Substance No.: 8134 Reference Substance No. (if applicable): —

PROTOCOL APPROVAL

Sean P. Gallagher — STUDY DIRECTOR — DATE 6/13/08

For HOK — LABORATORY MANAGEMENT — DATE 6/13/08

SPONSOR'S REPRESENTATIVE — DATE 28 Feb 2008

PROTOCOL NO.: 289/021508/DAP-LC2/OECD-OPPTS/SUB289

INTRODUCTION

Wildlife International, Ltd. will conduct a flow-through life-cycle toxicity test with the cladoceran, *Daphnia magna*, for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures outlined in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1300 (1); ASTM E 1193-97 *Standard Guide for Daphnia magna Life-Cycle Toxicity Tests* (2); and OECD Guideline 211: *Daphnia magna* Reproduction Test (3). Raw data for all work performed at Wildlife International, Ltd. and the original final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the effects of 1-Chlorododecane on the survival, reproduction and growth of the cladoceran, *Daphnia magna*, under flow-through test conditions, for a period of 21 days.

EXPERIMENTAL DESIGN

Daphnids will be exposed to a geometric series of at least five test concentrations, a negative (dilution water) control, and a solvent control for 21 days. Two replicate test chambers will be tested for each treatment and control group. Each replicate will contain two compartments with 5 daphnids each for a total of 20 daphnids per treatment and control group.

Nominal test concentrations will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information and will be added to this protocol by amendment. Each test concentration will be at least 50% of the next higher one unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate. Water samples from appropriate test chambers will be collected at specified intervals for analysis of the test substance. Results of the analyses will be used to calculate mean measured test concentrations.

To control bias, neonate daphnids will be impartially assigned to test compartments at test initiation. No other potential sources of bias are expected to affect the results of the study. Observations of the survival of the first-generation daphnids will be made daily. With the onset of reproduction, the number of young in each test compartment will be counted and young will be removed approximately three times per week. At the end of the test, growth of the first-generation daphnids will be measured. The results of the test will be used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The NOEC is defined as the highest concentration that produces no significant effect on survival, reproduction, or growth. The LOEC is defined as the lowest concentration that produces a significant effect on survival, reproduction, or growth. The maximum acceptable toxicant concentration (MATC) will be calculated as the geometric mean of the NOEC and LOEC. If possible, 21-day EC50 values will be calculated based on first-generation mortality/immobility and reproduction.

The test will be repeated if >20% of the control organisms appear to be immobilized or stressed; if surviving control daphnids produce an average of <60 young; or if any ephippia are produced by control daphnids.

MATERIALS AND METHODS

Test Substance

Information on the characterization of test substance, 1-Chlorododecane, is required by Good Laboratory Practice (GLP) Standards and Principles. Specific information regarding the test substance used in this study will be recorded in the raw data and summarized in the report. The Sponsor is responsible for providing Wildlife International, Ltd. verification that the test substance has been characterized according to GLPs prior to its use in the study. If verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers that cannot be appropriately disposed of by the laboratory. The laboratory will not dispose of any unused test substance without the Sponsor's authorization.

Wildlife International, Ltd.

Preparation of Stock and Test Concentrations

The test substance will be administered to the test organism in water. This route of administration was selected because it represents the most likely route of exposure to aquatic organisms. The test substance will be mixed directly with dimethyl formamide (DMF) to form a stock solution that subsequently will be added to dilution water. All stock solutions and test concentrations will be prepared on the basis of active ingredient (a.i.) rather than in terms of test substance as received from the Sponsor. A solvent control group will be included in the test in addition to a negative (dilution water) control. The concentration of the organic solvent will not exceed 0.1 mL/L, when possible. The solvent concentration in the solvent control will be equal to the highest solvent concentration in test chambers containing the test substance.

Test Organism

The cladoceran, *Daphnia magna*, has been selected as the test species for this study. Daphnids are representative of an important group of aquatic invertebrates, and have been selected for use in the test based upon past use history and ease of culturing in the laboratory. Daphnid neonates to be used in the test will be less than 24 hours old and will be obtained from cultures maintained at Wildlife International, Ltd., Easton, Maryland. The identity of the species will be verified by the supplier of the original culture or by Wildlife International, Ltd. using appropriate taxonomic keys such as Pennak (4).

Daphnids will be cultured in water from the same source and at approximately the same temperature as will be used during the test. Any changes in culture water temperature should be less than 3°C during any 12-hour period during holding. Daphnids in the cultures producing neonates for the test will be held for at least 10 days prior to collection of the neonates for testing. Neonates from daphnids that show signs of disease or stress will not be used as test organisms. Daphnids in holding that produce ephippia also will not be used to supply neonates for testing.

Daphnids in the cultures will be fed once daily. The diet will be a mixture of yeast, ~~Cerophyll®~~ cereal grass media, and trout chow (YCT), supplemented with a suspension of the freshwater green alga, *Pseudokirchneriella subcapitata*. Daphnids will be fed daily during the test using the same diet as used in the cultures. The frequency of feeding will be documented in the raw data and the final report. Specifications for acceptable levels of contaminants in daphnid diets have not been established. However, there are no

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known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

Adults in the culture will have produced an average of three young per adult per day over the 7-day period prior to the test. Neonates will be obtained for testing from at least three individual adults that have produced at least one prior brood, have produced young before Day 10, and have produced at least 9 young in the previous brood. Prior to test initiation, the neonates will be collected from cultures and transferred to small containers. The daphnids then will be impartially distributed to the test compartments and released below the water surface using a wide-bore pipette.

Dilution Water

Water used for the culturing and testing of daphnids will be obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.45 μm and passed through an ultraviolet sterilizer in order to remove microorganisms and fine particles. Water used for culturing and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as $CaCO_3$	145
Alkalinity, mg/L as $CaCO_3$	190
pH	8.1
Specific Conductance, μmhos/cm	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Total organic carbon (TOC) will be measured in the well water at least monthly. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. The range of hardness during this period should be ±10% of the mean hardness. Analyses are performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the most recent GLP-compliant analyses will be summarized in the final report.

Test Apparatus

All test apparatus will be prepared for use in this study by acid washing with 10% HCl, as appropriate. A continuous-flow diluter will be used to provide each concentration of the test substance, a negative (dilution water) control, and a solvent (DMF) control. A syringe pump, peristaltic pump, or a similar device will be used to deliver the test substance stock solutions and DMF for the solvent control to mixing chambers where the test substance and solvent will be mixed with dilution water. Peristaltic pumps, if used, will be calibrated prior to the test and at approximately weekly intervals thereafter. Syringe pumps, if used, will be calibrated prior to the test. The flow of dilution water into each mixing chamber will be controlled using rotameters. The rotameters will be calibrated prior to the test and verified and/or calibrated at least once a week during the test. After mixing, test solutions will be split to each replicate test chamber. The proportion of water split to each replicate will be checked prior to the test and approximately weekly thereafter to ensure that these flow rates vary by no more than ±10% of the mean of the two replicates.

The diluter will be adjusted so that each test chamber receives at least 5 volume additions of test solution every 24 hours. The delivery of test substance to test chambers will begin at least 4 hours prior to test initiation in order to verify that the target concentrations of the test substance have been achieved. The general operation of the diluter will be checked visually at least two times per day during the test and at least once at the beginning and end of the test.

Test chambers will be indiscriminately positioned in a temperature-controlled environmental chamber or water bath to maintain a temperature of 20 ± 1°C throughout the test period. The daphnids will be held in two test compartments suspended in each of two test chambers, for a total of four test compartments per concentration. The test chambers will be 25-L, Teflon®-lined or unlined, stainless steel aquaria filled with approximately 22 L of test solution. Test compartments will be constructed from glass beakers, approximately 6.5 cm in diameter and 12 cm in height. Nylon mesh screens will cover two holes on opposite sides of each test compartment to permit test solution to flow in and out of the compartment. Test chambers and compartments will be labeled with project number, test concentration and replicate.

Environmental Conditions

Lighting used to illuminate the cultures and test chambers during culturing and testing will be provided by fluorescent tubes that emit wavelengths similar to natural sunlight (e.g., Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark will be controlled with an automatic timer. A 30-minute transition of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity will be measured at test initiation with a SPER Scientific Ltd. light meter or equivalent.

The target test temperature will be 20 ± 1°C. Temperature will be measured in each test chamber at the beginning of the test and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also will be measured with a continuous recorder in one negative control chamber. Recorder measurements will be verified in the same control chamber with a liquid-in-glass thermometer prior to test initiation and at least weekly thereafter.

Dissolved oxygen will be measured in alternate test chambers of each treatment and control group at test initiation and termination, and approximately three times per week during the test (e.g., Monday, Wednesday and Friday) using a Thermo Orion Model 850Aplus dissolved oxygen meter, or equivalent. In the event that dissolved oxygen levels approach or fall below 60% saturation, the Sponsor will be notified, and appropriate corrective actions will be taken, if necessary, to maintain dissolved oxygen levels above approximately 60%. Measurements of pH will be made in alternate chambers of each treatment and control group at test initiation and at approximately weekly intervals thereafter using a Thermo Orion Model 525Aplus pH meter, or equivalent. When 100% mortality is observed in a test chamber, final measurements of dissolved oxygen, pH and temperature will be made.

Hardness, alkalinity, and specific conductance will be measured in alternate chambers of the negative (dilution water) control and the highest test concentration at test initiation and at approximately weekly intervals thereafter. During the test, the range of hardness in the negative (dilution water) control should be ±10% of the mean hardness. Total organic carbon (TOC) will be measured in the dilution water at test initiation and termination. Hardness and alkalinity measurements will be made by titration using procedures based on methods in *Standard Methods for the Examination of Water and Wastewater* (5). Specific conductance will be measured using a Yellow Springs Instrument Model 33 Salinity-

Conductivity-Temperature meter, or equivalent. TOC will be measured on a Shimadzu Model 5000 TOC analyzer, or equivalent. Additional water quality measurements may be taken as deemed necessary by study personnel. The reason for the additional measurements will be documented in the raw data and summarized in the final report.

Biological Observations

The first generation daphnids will be observed daily for survival, clinical signs of toxicity and the onset of reproduction. Immobilization is defined as a lack of movement except for minor spontaneous random movement of the appendages. Examples of clinical signs of toxicity include inability to maintain position in the water column, uncoordinated swimming and cessation of feeding. The presence of eggs in the brood pouch, aborted eggs, males, or ephippia also will be recorded daily. Observations of the onset of reproduction in each test compartment will be used to calculate the time to first brood, calculated as the number of days after test initiation until the offspring are first observed for each compartment.

With the onset of reproduction, the number of second generation daphnids will be counted in each test compartment approximately three times per week (e.g., Monday, Wednesday and Friday). Live first-generation daphnids will be retained and second-generation daphnids will be discarded. At the end of the test, the length and dry weight of each surviving first-generation daphnid will be measured.

Sampling for Analytical Measurements

Water samples will be collected from one alternating replicate test chamber of each treatment group prior to the test, at the beginning of the test, at approximately weekly intervals during the test, and at the end of the test to determine concentrations of the test substance. In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth from each test chamber, placed in an appropriate acid-washed storage container (e.g., glass or polypropylene bottle), extracted immediately and stored under refrigeration until analyzed, if necessary. The sample scheme is summarized below:

Wildlife International, Ltd.

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ESTIMATED NUMBERS OF SAMPLES

Experimental Group	Pre-test[a,b]	Day 0[a]	Day 7[a]	Day 14[a]	Day 21[a]
Control	1	1	1	1	1
Solvent Control	1	1	1	1	1
Level 1-Low Concentration	1	1	1	1	1
Level 2	1	1	1	1	1
Level 3	1	1	1	1	1
Level 4	1	1	1	1	1
Level 5-High Concentration	1	1	1	1	1
TOTALS	7	7	7	7	7

[a]One sample for each concentration, alternating between replicates for each sampling interval.

[b]Pre-test samples will be collected after conditioning of the diluter. More than one pre-test sampling interval may be required, depending upon the Sponsor's needs, and additional sampling and results will be documented in the raw data and included in the final report.

The above numbers of samples represent those that will be collected and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study. At the discretion of the Study Director, water samples from one or more appropriate test chambers and/or stock solutions will be collected and analyzed if an error in sampling or analysis is suspected, or if a malfunction in the test substance delivery system occurs. The reason for the additional samples will be described by the Study Director and documented in the raw data and final report.

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International, Ltd. The analytical method used will be based upon chromatographic methodology provided by the Sponsor and/or developed at Wildlife International, Ltd. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

Data Analyses

Test endpoints analyzed statistically for first generation daphnids will be survival, reproduction (the number of live young produced per reproductive day), and growth (length and dry weight). The data from the negative control and solvent control groups will be compared using an appropriate statistical test. If no statistical differences are found, then the data of the two control groups may be pooled. If

statistical differences are found, then the solvent control group will be used to evaluate the treatment-related effects.

Survival data will be analyzed using Chi-square and Fisher's Exact tests to identify treatment groups that show a statistically significant difference from the controls. Analyses of reproduction (number of young produced per reproductive day) and growth (weight and length) data will be evaluated for normality and homogeneity of variances. If the data are deemed normal with homogeneous variances, hypothesis testing using analysis of variance (ANOVA) and multiple means tests (e.g., Dunnett's, Bonferroni, Scheffe) will be used. If the data fail the tests for normality or homogeneity, then data transformations will be tried in an attempt to correct the condition. When the data transformations fail to correct for non-normality or heterogeneity of variances, nonparametric procedures may be used to identify statistically significant differences among the experimental groups. All statistical evaluations will be done using commercially available computer software such as TOXSTAT (6) or SAS (7).

The statistical analyses of survival, growth and reproduction data will aid but may not be exclusively used in the determination of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). The maximum acceptable toxicant concentration (MATC) will be calculated as the geometric mean of the NOEC and LOEC. When possible, 21-day EC50 values will be calculated based on first-generation mortality/immobility and reproduction (8, 9).

RECORDS TO BE MAINTAINED

Records to be maintained for data generated at Wildlife International, Ltd. will include, but will not be limited to:

1. The original signed protocol and protocol amendments and/or deviations (if any).
2. Identification and characterization of the test substance, as provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Length and dry weight measurements.
5. Reproduction parameters.
6. Test organism culture records.
7. Results of rangefinding tests, when applicable.
8. Calculation and preparation of stock solution and test concentrations.
9. Daily observations.

10. Water chemistry calculations (e.g., hardness and alkalinity).
11. Test conditions and physical/chemical measurements.
12. The methods used to analyze test substance concentrations, sample collection information and the results of analytical measurements.
13. Statistical calculations.
14. The original final report.

FINAL REPORT

A final report formatted to meet U.S. EPA's PR Notice 86-5 reporting requirements will be prepared by Wildlife International, Ltd. A draft report will be provided to the Sponsor's Representative for review prior to issuance of a final report. The report will include, but will not be limited to the following, when applicable:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures, as stated in the approved protocol, including changes in the original protocol.
5. The test substance identification, including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor. A copy of the Certificate of Analysis for the test substance, as provided by the Sponsor, will be included in the appendices.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor or contracted to Wildlife International Ltd.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source, scientific name, age, life stage, feed types, light intensity and photoperiod.
9. A description of the preparation of the test solutions, the methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, and the duration of the test.

10. The ranges and time-weighted average measured concentrations of dissolved oxygen (as percent saturation) and temperature during the test.
11. A description of circumstances that may have affected the quality or integrity of the data.
12. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
13. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
14. Statistical methods used to evaluate the data, and a concentration-response curve for first-generation mortality/immobility at the end of the test, when possible.
15. The signed and dated reports from the individual scientists or other professionals involved in the study.
16. The location where raw data and final report are to be stored.
17. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
18. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed and dated by the Study Director following review by the Sponsor's Representative.
19. A copy of the final protocol and protocol amendments (if any).

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director following approval by the Sponsor's Representative. The amendment will clearly identify the part(s) of the protocol to be amended, the reasons for the alteration(s) and the impact that the alteration(s) will have on the study. Amendments will be considered as part of the protocol and will be attached to the final protocol. Unplanned changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All deviations to the protocol will be indicated in the final report.

Wildlife International, Ltd.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. Raw data for all work performed at Wildlife International, Ltd. and the original final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

Wildlife International, Ltd.

REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1300: *Daphnid Chronic Toxicity Test*.

2 **ASTM.** 1997. *Standard Guide for Conducting Daphnia magna Life-Cycle Toxicity Tests*. American Society for Testing and Materials. Guideline E 1193-97.

3 **Organization for Economic Cooperation and Development.** 1998. Guideline 211: *Daphnia magna* Reproduction Test. OECD Guideline for Testing of Chemicals.

4 **Pennak, R.W.** 1978. *Freshwater Invertebrates of the United States*. 2nd Ed. 365 p.

5 **APHA, AWWA, WPCF.** 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

6. **West, Inc. and D.D. Gulley.** 1996. TOXSTAT® Version 3.5. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.

7. **The SAS System for Windows.** 2001. Version 8.2. SAS Institute Inc., Cary, North Carolina.

8. **Stephan, C.E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

9. **Finney, D.J.** 1971. *Statistical Methods in Biological Assay*. Second edition. Griffin Press, London.

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: A Flow-Through Life-Cycle Toxicity Study of 1-Chlorododecane with the Cladoceran (*Daphnia magna*)

PROTOCOL NO.: 289/021508/DAP-LC2/OECD-OPPTS/SUB289 **AMENDMENT NO.:** 1

SPONSOR: Lonza Inc. **PROJECT NO.:** 289A-166

EFFECTIVE DATE: June 13, 2008

AMENDMENT: Final Report Section, Page 12

Replace: A final report formatted to meet U.S. EPA's PR Notice 86-5 reporting requirements will be prepared by Wildlife International, Ltd.

With: A final report will be prepared by Wildlife International, Ltd.

REASON: The final report is not required to comply with EPA's PR Notice 86-5 format for the intended study submission(s).

IMPACT: This change has no impact on the integrity and validity of the study.

STUDY DIRECTOR — DATE 7/7/08

LABORATORY MANAGEMENT — DATE 7/7/08

SPONSOR'S REPRESENTATIVE — DATE 3 July 2008

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: A Flow-Through Life-Cycle Toxicity Study of 1-Chlorododecane with the Cladoceran (*Daphnia magna*)

PROTOCOL NO.: 289/021508/DAP-LC2/OECD-OPPTS/SUB289 **AMENDMENT NO.:** 2

SPONSOR: Lonza Inc. **PROJECT NO.:** 289A-166

EFFECTIVE DATE: July 15, 2008

AMENDMENT: Page 2

Change the proposed experimental start date from June 19, 2008 to July 22, 2008, and change the proposed experimental termination date from July 10, 2008 to August 12, 2008.

REASON: The experimental start was postponed from the originally scheduled start date due to additional analytical method development that was required.

IMPACT: This change has no impact on the integrity and validity of the study.

STUDY DIRECTOR — DATE 7/15/08

LABORATORY MANAGEMENT — DATE 7/15/08

SPONSOR'S REPRESENTATIVE — DATE 7/21/08

Appendix 2

Certificate of Analysis

CERTIFICATE OF ANALYSIS

Product: 1-Chlorododecane

Batch #: N6227945

PSL Reference No.: 070416-3R

Date of Analysis: June 29, 2007

Result:

1-Chlorododecane 97.42%

Approval: John M. Sheehy, B.A.. Analytical Services Product Safety Labs — Date 8/16/07

QA Release: Rhonda S. Krick, B.S. Quality Assurance Product Safety Labs — Date 08/17/07

PSL GLP Study # 22018

Appendix 3

Dilution Water Chemistry Parameters Measured
During the 4-Week Period Immediately Preceding the Test

	Mean	Range
Specific Conductance (µS/cm)	357 (n = 4)	355 – 360
Hardness (mg/L as $CaCO_3$)	136 (n = 4)	132 – 140
Alkalinity (mg as Ca CO_3)	178 (n = 4)	176 – 180
pH	8.2 (n = 4)	8.2 – 8.2
Total Organic Carbon (mg C/L)	<1 (n = 1)	--[1]

[1] TOC is measured monthly.

Appendix 4

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Pesticides and Organics [1]			
Component	Measured Concentration (µg/L)	Component	Measured Concentration (µg/L)
Aldrin	< 0.020	Gamma BHC – Lindane	< 0.010
Alpha BHC	< 0.010	Gamma Chlordane	< 0.010
Alpha Chlordane	< 0.010	Guthion (Azinphos-methyl)	< 3.0
Beta BHC	< 0.020	HCB	< 0.010
Bolstar	< 2.0	Heptachlor	< 0.010
Chlordane	< 0.50	Heptachlor Epoxide	< 0.010
Coumaphos	< 3.0	Kepone	< 0.20
Delta BHC	< 0.010	Malathion	< 2.0
Demeton-O	< 2.0	Merphos	< 6.0
Demeton-S	< 2.0	Methoxychlor	< 0.10
Diazinon	< 3.0	Methyl Parathion	< 2.0
Dichlorvos	< 3.0	Mevinphos	< 3.0
Dieldrin	< 0.020	Mirex	< 0.11
Disulfoton	< 2.0	Naled	< 2.0
Dursban (Chlorpyrifos)	< 2.0	o,p-DDD	< 0.020
Endosulfan I	< 0.010	o,p-DDE	< 0.020
Endosulfan II	< 0.020	o,p-DDT	< 0.020
Endosulfan Sulfate	< 0.020	p,p-DDD	< 0.020
Endrin	< 0.020	p,p-DDE	< 0.020
Endrin Aldehyde	< 0.10	p,p-DDT	< 0.020
Endrin Ketone	< 0.020	Phorate	< 2.0
EPN	< 2.0	Ronnel	< 2.0
Ethion	< 2.0	Stirophos	< 2.0
Ethoprop	< 3.0	Telodrin	< 0.010
Ethyl Parathion	< 2.0	Tokuthion	< 2.0
Famphur	< 2.0	Toxaphene	< 3.0
Fensulfothion	< 3.0	Trichloronate	< 2.0
Fenthion	< 2.0	Trithion	< 2.0

[1] Analyses performed by Lancaster Laboratories on samples collected on December 19, 2007.

Appendix 4 (Continued)

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Metals [1]			
Component	Measured Concentration (mg/L)	Component	Measured Concentration (mg/L)
Aluminum	< 0.200	Magnesium	14.6
Antimony	< 0.0200	Manganese	< 0.0050
Arsenic	< 0.0200	Mercury	< 0.00020
Barium	< 0.0050	Nickel	< 0.0100
Beryllium	< 0.0050	Nitrate Nitrogen	< 0.50
Bromide	< 2.5	Nitrite Nitrogen	< 0.50
Cadmium	< 0.0050	Potassium	6.97
Calcium	38.7	Selenium	< 0.0200
Chloride	4.2	Silver	< 0.0050
Chromium	< 0.0150	Sodium	19.8
Cobalt	< 0.0050	Sulfate	6.0
Copper	< 0.0100	Thallium	< 0.0200
Fluoride	0.55	Vanadium	< 0.0050
Iron	< 0.200	Zinc	< 0.0200
Lead	< 0.0150		

[1] Analyses performed by Lancaster Laboratories on samples collected on December 19, 2007.

Appendix 5

The Analysis of 1-Chlorododecane in Freshwater

Appendix 5.1

Analytical Method Flowchart for the Analysis of 1-Chlorododecane in Freshwater

METHOD OUTLINE FOR THE ANALYSIS OF 1-CHLORODODECANE IN FRESHWATER

Rinse separatory funnels with ethyl acetate.

↓

Transfer the requisite volume of freshwater to the separatory funnels. Fortify recovery samples with the appropriate stock solution(s).

↓

Using a graduated cylinder or tiltapet, add the requisite volume of ethyl acetate to each sample. Stopper and shake each sample (with venting) for approximately one minute. Allow the organic and aqueous layers to separate. Drain the aqueous phase to waste.

↓

Using a disposable pipette, transfer an aliquot of the ethyl acetate extract to a scintillation vial containing a few grains of sodium sulfate. Swirl the extract to ensure removal of residual water.

↓

Transfer an aliquot of each extract to an autosampler vial. Submit samples for GC/MS analysis.

Appendix 5.2

Typical Gas Chromatograph (GC) Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 5890 Gas Chromatograph (GC)
DETECTOR:	Hewlett-Packard Model 5971A Mass Selective Detector operated in Selective Ion Monitoring (SIM) mode
ANALYTICAL COLUMN:	ZB-5 column (30 m x 0.25 mm ID, 0.25 µm)
INJECTOR TEMPERATURE:	250ºC
OVEN:	Initial temperature: 50°C Initial hold time: 4.00 minute Ramp: 35.00°C/minute Final temperature: 340°C Final hold time: 0.00 minute
DETECTOR TEMPERATURE:	300°C
CARRIER GAS:	Helium
HEAD PRESSURE:	12 psi
INJECTION VOLUME:	2 µL
ION MONITORED:	91.00[1]
APPROXIMATE 1-CHLORODODECANE RETENTION TIME:	9.5 minutes

[1] The fragment ion 91 m/z (C_4H_8C1+) was selected to maintain sensitivity and selectivity, provided by its relatively high mass.

Appendix 5.3

Analytical Stocks and Standards Preparation

Stock solutions of 1-Chlorododecane were prepared by weighing 0.1027 grams (weight corrected for purity) of the test substance on an analytical balance. The test substance was transferred to a 100-mL class A volumetric flask brought to volume using acetone. The primary stock solution (1.00 mg a.i./mL) was serially diluted in acetone to prepare 0.100, 0.0100 and 0.00100 mg a.i./mL stock solutions. The 0.00100 and 0.0100 mg a.i./mL stock solutions were used to fortify the quality control samples. Calibration standards were prepared in ethyl acetate using the 0.0100 mg a.i./mL stock solution*. The following shows the dilution scheme for a set of calibration standards:

Stock Concentration (mg a.i./mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (µg a.i./L)
0.0100	60.0	100	6.00
0.0100	150	100	15.0
0.0100	300	100	30.0
0.0100	450	100	45.0
0.0100	600	100	60.0

* A 4.50 µg a.i./L standard was required. The 45.0 µg a.i./L standard was diluted 10.0 mL to 100 mL in ethyl acetate to prepare the 4.50 µg a.i./L stock solution.

Appendix 5.4

Example Calculations for a Representative Sample

The analytical result and percent recovery for sample number 289A-166-3, with a nominal concentration of 6.3 µg a.i./L, were calculated using the following equations:

$$\text{Concentration of 1-Chlorododecane in sample (µg a.i./L)} = \frac{\text{peak area - (y-intercept)}}{\text{slope}} \text{ x dilution factor}$$

$$\text{Percent of nominal concentration} = \frac{\text{Measured concentration of sample (µg a.i./L)}}{\text{Nominal concentration of sample (µg a.i./L)}} \text{ x } 100$$

Peak Area = 8752
Y-Intercept = -2319.20922
Slope = 2208.15992
Dilution Factor = 0.500

$$\text{Concentration of 1-Chlorododecane in sample (µg a.i./L)} = \frac{8752 + 2319.20922}{2208.15992} \text{ x } 0.500$$

Concentration of 1-Chlorododecane in sample (µg a.i./L) = 2.51

$$\text{Percent of nominal concentration} = \frac{2.51 \text{ µg a.i./L}}{6.3 \text{ µg a.i./L}} \text{ x } 100$$

Percent of nominal concentration = 39.8%

Appendix 5.5

Quality Control Samples of 1-Chlorododecane in Freshwater

Sample Number (289A-166-)	Sample Type	Fortified Concentration (µg a.i./L)	Measured Concentration (µg a.i./L)	Percent Recovery[1]
Pre-Test				
PTMAB-1	Matrix Blank	0.0	< LOQ[2]	--
PTMAS-1	Matrix Fortification	6.00	6.62	110
PTMAS-2	Matrix Fortification	25.0	24.2	96.7
PTMAS-3	Matrix Fortification	100	83.7	83.7
Exposure				
MAB-1	Matrix Blank	0.0	< LOQ[3]	--
MAB-2	Matrix Blank	0.0	< LOQ[3]	--
MAB-3	Matrix Blank	0.0	< LOQ[3]	--
MAB-4	Matrix Blank	0.0	< LOQ[3]	--
MAS-1	Matrix Fortification	6.00	5.56	92.6
MAS-2	Matrix Fortification	25.0	22.1	88.2
MAS-3	Matrix Fortification	100	80.0	80.0
MAS-4	Matrix Fortification	6.00	6.09	101
MAS-5	Matrix Fortification	25.0	23.7	94.6
MAS-6	Matrix Fortification	100	75.1	75.1
MAS-7	Matrix Fortification	6.00	6.82	114
MAS-8	Matrix Fortification	25.0	26.9	108
MAS-9	Matrix Fortification	100	95.1	95.1
MAS-10	Matrix Fortification	6.00	6.76	113
MAS-11	Matrix Fortification	25.0	27.1	109
MAS-12	Matrix Fortification	100	86.6	86.6
			Mean =	96.4
			Standard Deviation =	12.8
			CV =	13.3%

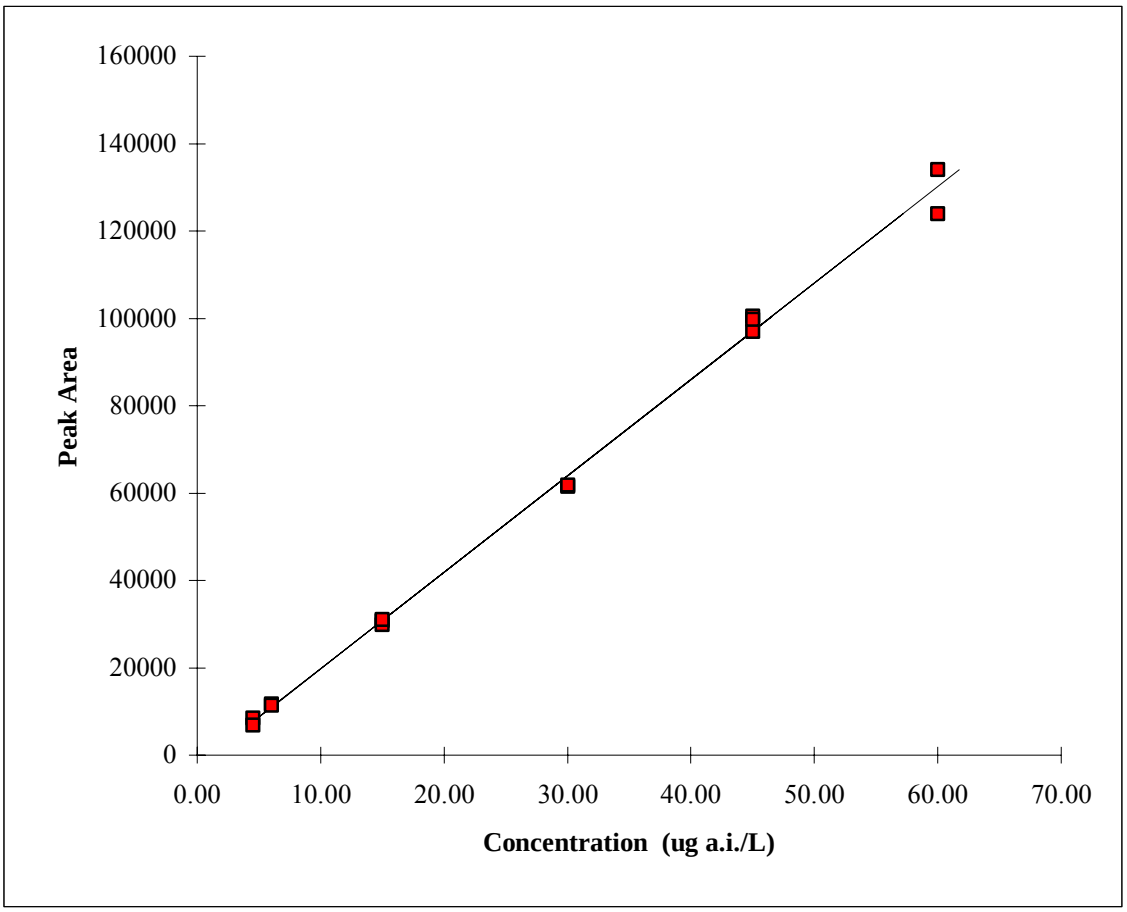
[1] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for the pre-test samples was 3.00 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (6.00 µg a.i./L) and the dilution factor of the matrix blank samples (0.500).

[3] The limit of quantitation (LOQ) for the test samples was 2.25 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (4.50 µg a.i./L) and the dilution factor of the matrix blank samples (0.500).

Appendix 5.6

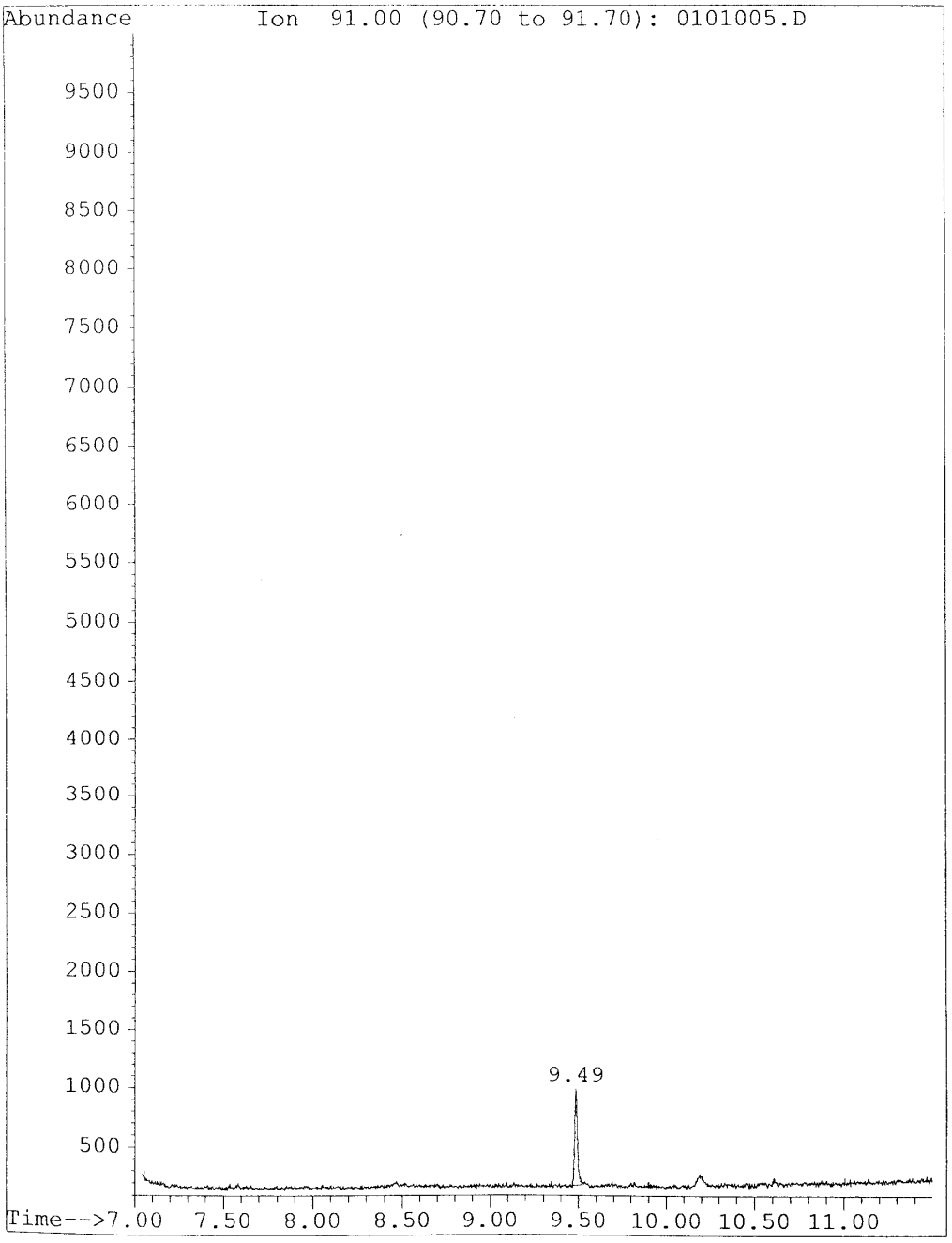
Representative Calibration Curve for 1-Chlorododecane



Slope = 2208.15992; Y-Intercept = -2319.20922; R^2 = 0.99680

Appendix 5.7

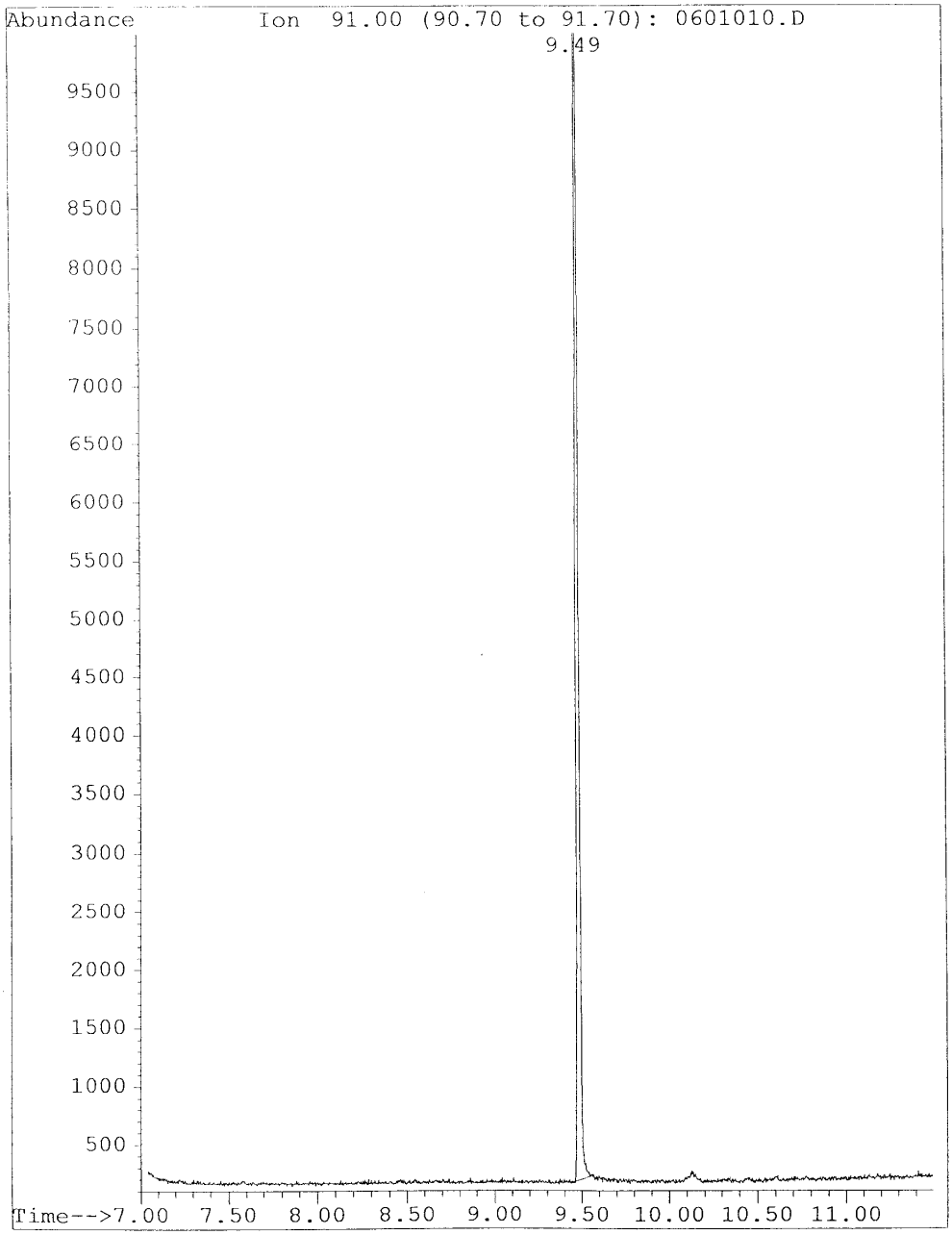
Representative Chromatogram of a Low-level 1-Chlorododecane Calibration Standard



Nominal concentration: 4.50 µg a.i./L

Appendix 5.8

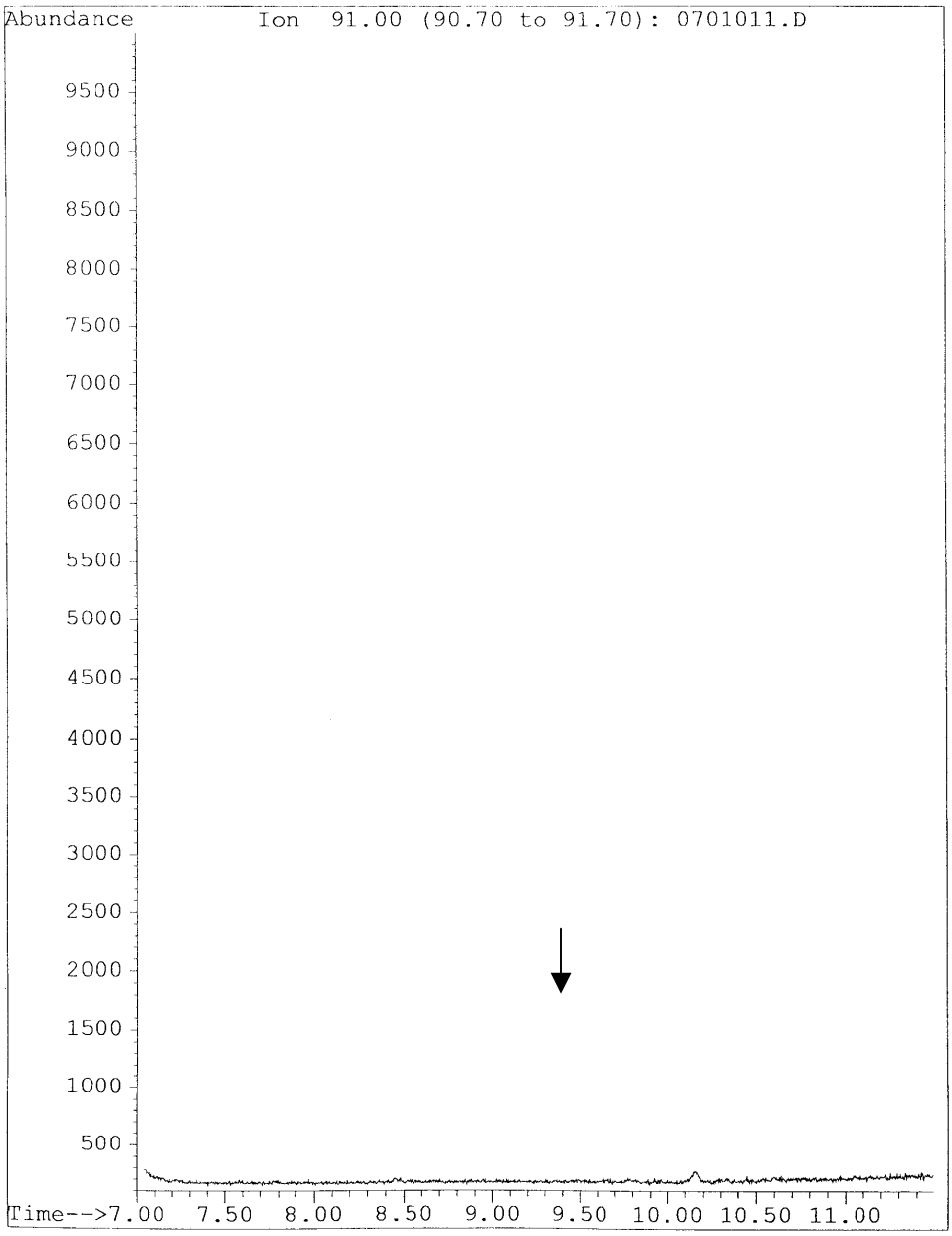
Representative Chromatogram of a High-level 1-Chlorododecane Calibration Standard



Nominal concentration: 60.0 µg a.i./L

Appendix 5.9

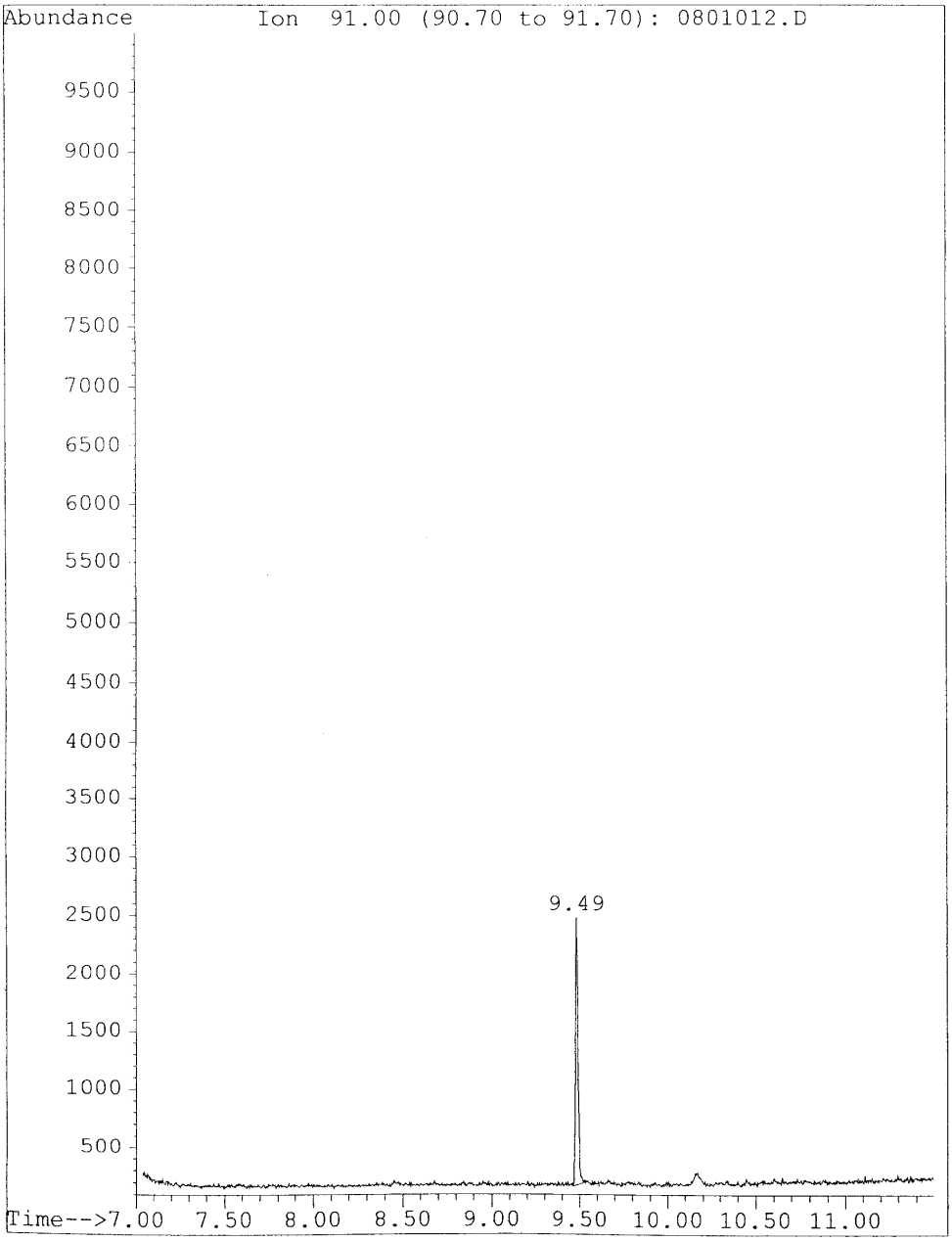
Representative Chromatogram of a Matrix Blank Sample



Sample number 289A-166-MAB-1; Day 0. The arrow indicates the approximate retention time of 1-Chlorododecane.

Appendix 5.10

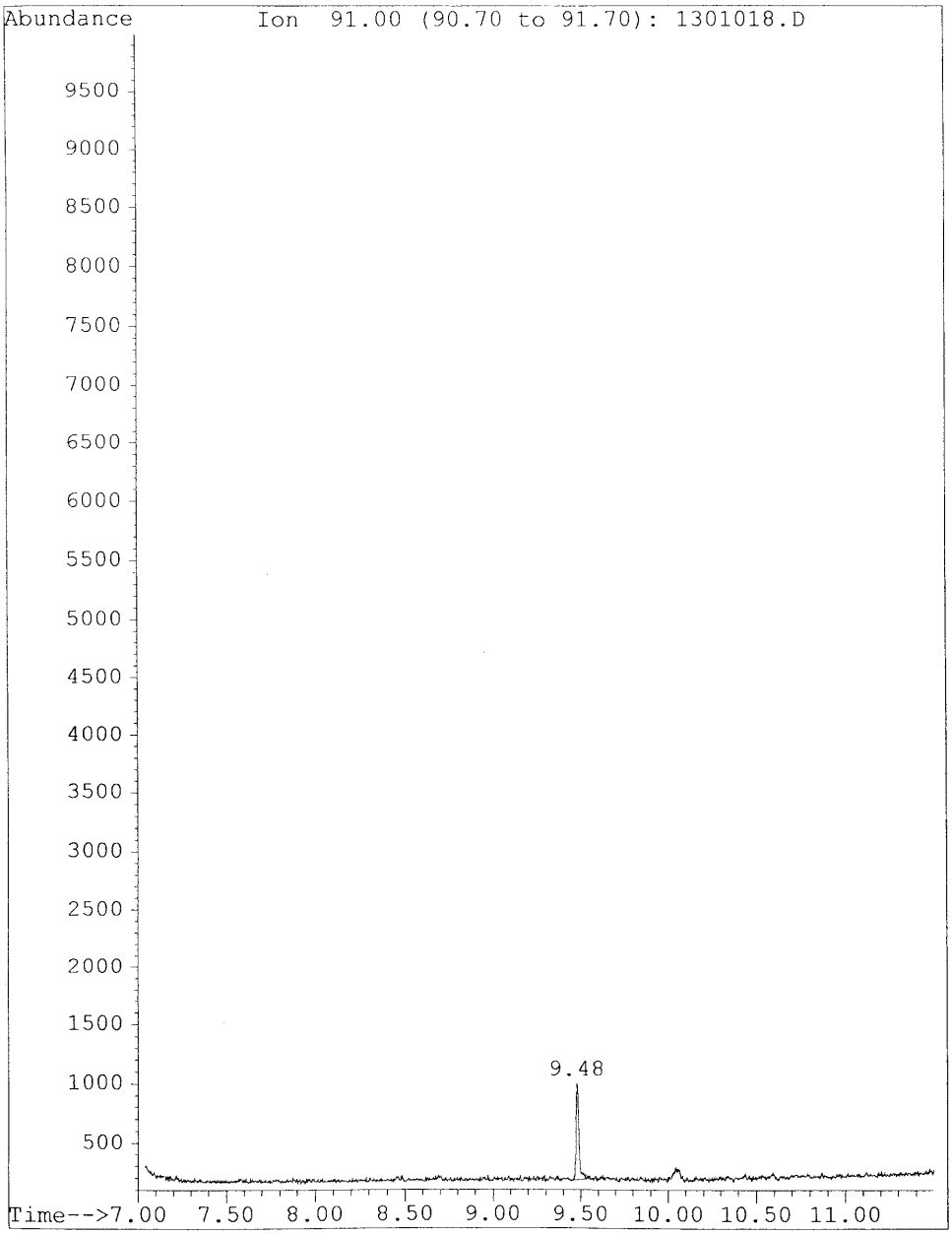
Representative Chromatogram of a Matrix Fortification Sample



Sample number 289A-166-MAS-1; Nominal concentration: 6.00 µg a.i./L

Appendix 5.11

Representative Chromatogram of a Test Sample



Sample number 289A-166-3; Day 0; Nominal concentration: 6.3 µg a.i./L

Appendix 6

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	Negative Control											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+; 1 AN
6 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
7	0	0	5 AN+; P	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+; P
8 (T)	0	0	5 AN+	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
12	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
13 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
17	0	0	5 AN+	1	0	4 AN+	0	0	5 AN+	0	0	5 AN+
18 (T)	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+	0	0	5 AN+
19	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+	0	0	5 AN+
20	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+	0	0	5 AN+
21	0	0	4 AN+; 1 AN	0	0	4 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	Solvent Control											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	5 AN+	0	0	3 AN+; 2 AN	0	0	4 AN+; 1 AN	0	0	3 AN+; 2 AN
6 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+; 1 AN
7	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P
8 (T)	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
9	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
12	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+; 1 AN
13 (T)	0	0	5 AN+	1	0	4 AN+	0	0	5 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	4 AN+	0	0	4 AN+; 1 D+	1	0	4 AN+
17	0	0	5 AN+	0	0	4 AN+	0	0	4 AN+; 1 D+	0	0	4 AN+
18 (T)	0	0	5 AN+	0	0	4 AN+	1	0	4 AN+	0	0	4 AN+
19	0	0	5 AN+	0	0	4 AN+	0	0	4 AN+	0	0	4 AN+
20	0	0	5 AN+	0	0	4 AN+	0	0	4 AN+	0	0	4 AN+
21	0	0	5 AN+	0	0	4 AN+	0	0	4 AN+	0	0	4 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; D = discoloration (pale); P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	1.5 µg a.i./L											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	1 AN+; 4 AN	0	0	3 AN+; 2 AN	0	0	5 AN+	0	0	5 AN+
6 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
7	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+
8 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+; P
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
12	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
13 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
17	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
18 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
19	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
20	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
21	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	2.7 µg a.i./L											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
6 (T)	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
7	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
8 (T)	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
12	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
13 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+
17	0	0	5 AN+	0	0	3 AN+; 2 AN	0	0	5 AN+	0	0	5 AN+
18 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
19	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
20	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
21	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	5 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	5.7 µg a.i./L											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	4 AN+; 1 AN	0	0	3 AN+; 2 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN
6 (T)	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN
7	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
8 (T)	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+; P
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	1	0	4 AN+	0	0	5 AN+
12	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
13 (T)	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
17	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
18 (T)	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
19	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
20	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
21	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	7.2 µg a.i./L											
	Compartment A			Compartment B			Compartment C			Compartment D		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	4 AN; 1 Q, AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	5 AN+
6 (T)	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	5 AN+
7	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
8 (T)	0	0	5 AN+; P	0	0	5 AN+; P	0	0	5 AN+	0	0	5 AN+; P
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
11 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+; P	0	0	5 AN+
12	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+; 1 C+	0	0	5 AN+
13 (T)	0	0	5 AN+	0	0	5 AN+	1	0	4 AN+	0	0	5 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
15 (T)	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
16	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
17	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
18 (T)	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
19	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
20	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+
21	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+	0	0	5 AN+

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; Q, AN = daphnid floating, but appears normal after gentle submersion; C = lethargy; + = eggs present in brood pouch; P = onset of production.

Appendix 6 (Continued)

Mortality and Clinical Observations of First-Generation Daphnids

Day of Test[1]	17 µg a.i./L											
	Compartment A			Compartment B			Compartment C[4]			Compartment D[4]		
	# Dead	# Imm.[2]	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]	# Dead	# Imm.	Observations[3]
0	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
1	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
2	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
3	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
4 (T)	0	0	5 AN	0	0	5 AN	0	0	5 AN	0	0	5 AN
5	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	1 AN+; 4 AN	0	0	4 AN+; 1 AN
6 (T)	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	4 AN+; 1 AN	0	0	5 AN+
7	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+
8 (T)	0	0	5 AN+	0	0	5 AN+; P	0	0	5 AN+; P	1	0	4 AN+; P
9	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
10	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
11 (T)	0	0	5 AN+; P	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
12	0	0	5 AN+	0	0	4 AN+; 1 AN	0	0	5 AN+	0	0	3 AN+; 1 AN
13 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
14	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
15 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
16	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
17	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
18 (T)	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
19	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
20	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	4 AN+
21	0	0	5 AN+	0	0	5 AN+	0	0	5 AN+	0	0	3 AN+; 1 AN

[1] T = Surviving adult daphnids were transferred to clean test chambers.
[2] Imm. = Immobile.
[3] Observations: AN = appear normal; + = eggs present in brood pouch; P = onset of production.
[4] At transfer on Day 13, first-generation daphnids from test compartment C were inadvertently returned to test compartment D, and first-generation daphnids from test compartment D were returned to test compartment C. While daphnids remained in these compartments for the remainder of the test, data are reported here in terms of test compartment as originally assigned.

Appendix 7

Neonate Production by First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Comp.	Neonate Production by Test Day [1,2]						Number Young Produced	Number of Reproductive Days[3]	Number Young Produced Per Reproductive Day
		8	11	13	15	18	21			
Negative Control	A	59	147	2	174	157	117	656	75	8.7
	B	48	142	0	115	135	128	568	70	8.1
	C	63	168	0	89	109	150	579	75	7.7
	D	59	184	0	144	110	123	620	75	8.3
Solvent Control	A	63	135	0	155	160	84	597	75	8.0
	B	39	196	34	116	119	116	620	66	9.4
	C	48	195	39	148	158	91	679	71	9.6
	D	47	196	37	137	130	98	645	69	9.3
1.5	A	40	178	45	140	155	118	676	75	9.0
	B	43	166	40	141	142	96	628	75	8.4
	C	48	171	3	204	194	103	723	75	9.6
	D	61	185	0	211	206	122	785	75	10.5
2.7	A	44	171	34	145	155	113	662	75	8.8
	B	44	225	44	166	174	113(2)[2]	766	75	10.2
	C	59	197	0	161	151	100	668	75	8.9
	D	72	153	1	151	158	129	664	75	8.9
5.7	A	39	120	40	131	184	133	647	75	8.6
	B	44	172	41	143	158	158	716	75	9.5
	C	44	140	40	103	123	117	567	64	8.9
	D	51	162	26	121	191	140	691	75	9.2
7.2	A	45	148	39	153	188	145	718	75	9.6
	B	41	148	42	165	195	144	735	75	9.8
	C	0	145	0	146	140	114	545	66	8.3
	D	2	173	44	161	202	153	735	75	9.8
17	A	0	48	34	94	142	113	431	75	5.7
	B	6	139	42	139	151	121	598	75	8.0
	C[4]	19	103	42	153	172	103	592	75	7.9
	D[4]	36	110	44	124	152	98	564	61	9.2

[1] The onset of neonate production for the control and treatment groups was observed on Days 7 and 8 of the test.
[2] (#) = number of immobile neonates produced.
[3] A reproductive day is defined as each day a first generation daphnid is alive after the onset of reproduction (e.g. 5 first-generation daphnids alive for one day would represent 5 reproductive days).
[4] At transfer on Day 13, first-generation daphnids from test compartment C were inadvertently returned to test compartment D, and first-generation daphnids from test compartment D were returned to test compartment C. While daphnids remained in these compartments for the remainder of the test, data are reported here in terms of test compartment as originally assigned.

Appendix 8

Length and Dry Weight of Surviving First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	Daphnid Number	Individual Length (mm)	Mean Length (mm)	Individual Dry Weight (mg)	Mean Dry Weight (mg)
Negative Control	A	1	5.6	5.5	1.04	1.07
		2	5.3		1.09	
		3	5.5		1.02	
		4	5.4		1.21	
		5	5.5		0.99	
	B	1	5.8	5.6	1.02	1.07
		2	5.7		1.10	
		3	5.5		1.00	
		4	5.4		1.14	
		5	-		-	
	C	1	5.5	5.6	0.96	0.99
		2	5.6		1.01	
		3	5.5		1.10	
		4	5.7		0.90	
		5	5.7		0.98	
	D	1	5.6	5.6	1.22	1.17
		2	5.5		1.19	
		3	5.6		1.25	
		4	5.5		1.11	
		5	5.7		1.09	
Solvent Control	A	1	5.4	5.3	0.84	0.91
		2	5.4		0.84	
		3	5.4		0.97	
		4	5.3		1.05	
		5	5.1		0.87	
	B	1	5.4	5.6	1.03	1.02
		2	5.8		1.07	
		3	5.6		0.95	
		4	5.5		1.04	
		5	-		-	
	C	1	5.5	5.5	1.10	1.00
		2	5.3		0.99	
		3	6.0		1.09	
		4	5.3		0.81	
		5	-		-	
	D	1	5.6	5.5	1.13	1.04
		2	5.5		1.07	
		3	5.6		1.03	
		4	5.3		0.93	
		5	-		-	

Appendix 8 (Continued)

Length and Dry Weight of Surviving First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	Daphnid Number	Individual Length (mm)	Mean Length (mm)	Individual Dry Weight (mg)	Mean Dry Weight (mg)
1.5	A	1	5.6	5.4	1.01	1.00
		2	5.5		0.82	
		3	5.3		0.94	
		4	5.5		1.00	
		5	5.3		1.25	
	B	1	5.2	5.5	0.86	1.12
		2	5.6		1.05	
		3	5.3		1.25	
		4	5.8		1.16	
		5	5.6		1.26	
	C	1	5.0	5.5	1.15	1.09
		2	5.7		1.03	
		3	5.4		1.08	
		4	5.7		1.24	
		5	5.5		0.95	
	D	1	5.7	5.7	1.06	1.08
		2	5.8		1.02	
		3	5.5		1.15	
		4	5.8		1.03	
		5	5.7		1.12	
2.7	A	1	5.3	5.2	0.81	0.91
		2	5.4		0.98	
		3	5.0		1.06	
		4	5.5		0.84	
		5	4.9		0.85	
	B	1	5.6	5.7	0.91	0.97
		2	5.8		1.01	
		3	5.8		1.02	
		4	5.7		1.00	
		5	5.4		0.90	
	C	1	5.8	5.6	1.00	0.92
		2	5.3		0.88	
		3	5.6		0.84	
		4	5.6		1.00	
		5	5.5		0.89	
	D	1	5.5	5.5	0.97	0.98
		2	5.6		0.98	
		3	5.5		0.94	
		4	5.7		1.04	
		5	5.2		0.95	

Appendix 8 (Continued)

Length and Dry Weight of Surviving First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	Daphnid Number	Individual Length (mm)	Mean Length (mm)	Individual Dry Weight (mg)	Mean Dry Weight (mg)
5.7	A	1	5.5	5.7	1.03	1.09
		2	5.6		1.11	
		3	6.0		1.16	
		4	5.8		1.19	
		5	5.5		0.95	
	B	1	5.5	5.6	1.04	1.01
		2	5.6		0.97	
		3	5.6		0.92	
		4	5.5		0.91	
		5	5.6		1.19	
	C	1	5.8	5.7	1.36	1.12
		2	5.5		1.09	
		3	5.7		1.01	
		4	5.8		1.03	
		5	-		-	
	D	1	5.4	5.5	1.32	1.08
		2	5.4		1.04	
		3	5.1		1.06	
		4	5.9		1.15	
		5	5.6		0.81	
7.2	A	1	5.7	5.6	1.02	1.06
		2	5.4		1.17	
		3	5.6		1.12	
		4	5.4		1.06	
		5	5.7		0.94	
	B	1	5.6	5.6	0.99	1.03
		2	5.7		0.96	
		3	6.0		1.04	
		4	5.0		0.99	
		5	5.5		1.15	
	C	1	5.7	5.7	0.71	0.98
		2	5.8		1.08	
		3	5.5		1.23	
		4	5.8		0.90	
		5	-		-	
	D	1	5.6	5.7	0.84	0.97
		2	5.6		1.30	
		3	5.8		0.89	
		4	5.8		0.88	
		5	5.8		0.93	

Appendix 8 (Continued)

Length and Dry Weight of Surviving First-Generation Daphnids

Mean Measured Concentration (µg a.i./L)	Compartment	Daphnid Number	Individual Length (mm)	Mean Length (mm)	Individual Dry Weight (mg)	Mean Dry Weight (mg)
17	A	1	4.9	4.9	0.81	0.87
		2	4.8		0.69	
		3	5.1		0.99	
		4	5.1		0.93	
		5	4.6		0.93	
	B	1	5.3	5.2	0.82	0.92
		2	5.3		0.79	
		3	5.1		0.92	
		4	4.7		1.00	
		5	5.6		1.06	
	C[1]	1	5.3	5.3	0.91	1.00
		2	5.2		0.92	
		3	5.3		1.12	
		4	5.2		1.13	
		5	5.3		0.92	
	D[1]	1	5.7	5.3	1.07	0.95
		2	5.3		0.85	
		3	5.1		0.85	
		4	5.1		1.03	
		5	-		-	

[1] At transfer on Day 13, first-generation daphnids from test compartment C were inadvertently returned to test compartment D, and first-generation daphnids from test compartment D were returned to test compartment C. While daphnids remained in these compartments for the remainder of the test, data are reported here in terms of test compartment as originally assigned.

Appendix 9

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. Henry O. Krueger, Ph.D., Director of Aquatic, Plant and Insect Toxicology
2. Willard B. Nixon, Ph.D., Director of Chemistry
3. Amy S. Blankinship, Laboratory Supervisor
4. Timothy Z. Kendall, Supervisor
5. Sean P. Gallagher, Senior Biologist
6. Mary Beth Claude, Biologist