Data Evaluation Report on the Acute Toxicity of Dimethyl Disulfide to Freshwater Invertebrates - *Daphnia* sp.

PMRA Submission Number {.....} EPA MRID Number 470528-16

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D338050
	OECD Data Point	{.....}
	EPA MRID	470528-16
	EPA Guideline	OPPTS 850.1010 (72-2a)

Test material: Dimethyl Disulfide TC **Purity:** 998.89 g/kg (99.9%)
Common name: DMDS
Chemical name: IUPAC: Dimethyl Disulfide
CAS name: Dimethyl Disulfide
CAS No.: 624-92-0
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 05/07/0

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 06/04/07

Secondary Reviewer: James Felkel 10/30/08 **Date:** {.....}
{EPA/OECD/PMRA}

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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Use Site Category:	{.....}	[For PMRA]
EPA PC Code	029088	

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Noack, Martina. 2007. Dimethyl Disulfide TC: Acute Immobilization Test (Semi-Static, 48 H) to *Daphnia magna*. Unpublished study performed by Dr. U Noack-Laboratorien, Kathe-Paulus-Str 1, D-31157 Sarstedt. Laboratory report number DAI106301. Study sponsored by Arkema/Thiochemistry Business Unit, Departement Securite Environnement Produit, France. Study completed January 8, 2007.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to freshwater invertebrates. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

This Data Evaluation Record may have been revised by the Environmental Fate and Effects Division subsequent to signing by Cambridge Environmental Inc. personnel.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Dimethyl Disulfide to *Daphnia magna* was studied under static-renewal conditions. Daphnids were exposed to nominal concentrations of 0 (negative control), 0.970, 2.13, 4.70, 10.3, 22.7, and 50 mg/L; time-weighted average (TWA) concentrations were <0.010 (<LOQ; negative control), 0.621, 1.79, 3.78, 8.27, 17.4 and 45.0 mg ai/L. Mortality and sub-lethal effects were observed daily. The 48-hour LC_{50}/EC_{50} was 1.61 mg ai/L. The 48-hr NOAEC based on immobility was 0.621 mg ai/L. With the exception of immobility, no other biological effects were reported.

Based on the results of this study, Dimethyl Disulfide would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

The study is considered to be scientifically sound and is categorized Acceptable. It is thus considered to satisfy guideline requirements for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (e.g., 1st instar): 2-24 Hours
Test Type (Flow-through, Static, Static Renewal): Static-Renewal

LC_{50}: 1.61 mg ai/L 95% C.I.: 1.31-1.99 mg ai/L
NOAEC: 0.621 mg ai/L
EC_{50}: Not Determined 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
Endpoint(s) Affected: Immobility

I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: This study was reported to be conducted following guidelines outlined in EPA OPPTS Draft Guideline No. 850.1010 (1996); OECD Guideline for Testing of Chemicals No. 202 (2004); and Directive 92/69/EC Method C.2 (1992) and that these guidelines are "in accordance" with the Japanese (METI) guideline.

The following deviations from OPPTS 850.1010 are noted in this review:

1. The source of the dilution water was not provided.
2. The hardness of the dilution water (167-167 mg/L as $CaCO_3$) was higher than U.S. EPA recommendations (40-48 mg/L as $CaCO_3$), but was acceptable according to OECD guidelines (140-250 mg/L as $CaCO_3$).
3. The results from a periodic screening analysis of the dilution water were not reported.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided; the study was declared to be conducted in compliance with the present OECD, EC and German principles of Good Laboratory Practice.

A. REPORTED MATERIALS:

1. Test material Dimethyl Disulfide TC

Description: Light Yellow Liquid

Lot No./Batch No. : 17-08-04 (Batch Number)

Purity: 998.89 g/kg (99.9%)

Stability of compound under test conditions: Analytical verification was conducted in all replicate vessels at 0 hours (new), 24 hours (new and aged) and 48 hours (aged). Recoveries in the new solutions at 0 hours were <70% of nominal in the all replicates of the nominal 0.970 mg/L treatment level, in one replicate of the nominal 4.70 mg/L treatment level and in two replicates of the nominal 50.0 mg ai/L treatment level. Recoveries in the aged solutions were >70% in all replicates of the nominal 0.970, 2.13, 4.70 and 50.0 mg/L treatment levels and <70% in all replicates of the nominal 10.3 and 22.7 mg/L treatment levels. The recoveries in the new solutions at 24 hours and the aged solutions at 48 hours were >70% in all replicates of the nominal 2.13-50.0 mg/L treatment levels; recoveries were <70% in all replicates at both intervals in the nominal 0.970 mg/L treatment level.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of

test chemicals: Room temperature, protected from moisture and light.

Physicochemical properties of Dimethyl Disulfide.

Parameter	Values	Comments
Water solubility at 20EC	Insoluble	
Vapor pressure	20°C: 28 hPa (mbar) 25°C: 38 hPa (mbar)	
UV absorption	Not Reported	
PKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: *Daphnia magna*
(EPA preferred species is *Daphnia magna;* OECD preferred species is *Daphnia magna* or any other suitable *Daphnia* species)

Age at test initiation: 2-24 Hours
(EPA recommends that Daphnids are in their first instar (<24 hrs old) and that all organisms are approximately the same size and age; OECD requires age <24 hrs old)

Source: In-house laboratory cultures. Daphnid culture did not contain ephippia, produced young before Day 12, exhibited <20% mortality 48 hours prior to test initiation and produced an average of 3 young per adult per day over a 7-day period prior to test initiation.
(EPA requires that all organisms are from the same source. Daphnids from ephippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent)

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 48-hour static-renewal range-finding study was conducted using nominal concentrations of 0 (negative control), 0.50, 5.00 and 50.0 mg/L. By test termination, immobility was 0% in the control and 0.50 mg/L treatment group and 95 and 100% in the 5.00 and 50.0 mg/L treatment groups, respectively.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 Continuous Same as test *Desmodesmus subspicatus* and *Chlorella vulgaris* (>10^6 cells/mL) was provided 5 times per week ad libitum. Normal and healthy.	 *The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Duration of the test	48 Hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test condition Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	 Static Renewal N/A Test solution was renewed after 24 hours	 *The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	Not Reported	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass Not Reported 130 mL	 *EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Source not reported. The dilution water was prepared according to Directive 92/69/EC Method C.2 Annex, Modified according to EPA.	*Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafi/850.1010Opdf). Dilution water should be intensely aerated before the study.*

Parameter	Details	Remarks *Criteria*
<u>Water parameters</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 167-169 mg/L as $CaCO_3$ 7.79-8.04 (new solutions) 7.70-7.93 (old solutions) ≥82% saturation 20.0-21.6°C Not Reported Not Reported Not Reported Not Reported Not Reported	 <u>*Hardness*</u>*:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* <u>*pH:*</u> *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* <u>*Temperature*</u>*:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) <u>*Dissolved oxygen*</u>*:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
<u>Number of replicates</u> Negative control: Treatments:	 4 4	 *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Negative control: Treatments:	 5 5	*EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing ∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17°C and ≤5 g/L at > 17°C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations Nominal: Measured:	0 (negative control), 0.970, 2.13, 4.70, 10.3, 22.7 and 50.0 mg/L <0.010 (<LOQ; negative control), 0.621, 1.79, 3.78, 8.27, 17.4 and 45.0 mg ai/L	The measured values represent the reviewer-calculated time-weighted average concentrations. *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	N/A; a solvent was not used	*Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D	*EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.*

Parameter	Details	Remarks *Criteria*
Stability of chemical in the test system	Variable. TWA recoveries were 77-90% of nominal for the nominal 2.13-50.0 mg/L treatment levels and the recovery of the TWA concentration was 64% of nominal for the nominal 0.970 mg/L treatment level.	There was no clear pattern of decline of the test material at any treatment level. The lowest treatment group exhibited an increase in the measured concentrations from 0-24 hours and from 24-48 hours. Additionally, the matrix fortifications yielded mean recoveries of 89-102% of nominal indicating accurate methods.
Recovery of chemical Level of Quantitation Level of Detection	 0.010 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations}	Potassium dichromate 1.66, 1.99, 2.39 and 2.87 mg/L	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sub-lethal effects	Immobility	
Observation intervals	24 and 48 hours	
Were raw data included?	Yes	
Other observations, if any	All test solutions were clear and colorless throughout the definitive exposure period.	

II. RESULTS AND DISCUSSION:

A. REPORTED MORTALITY:

By test termination, immobility/mortality was 0% in the negative control, and 5, 45, 100, 100, 100 and 100% in the TWA 0.621, 1.79, 3.78, 8.27, 17.4 and 45.0 mg ai/L treatment groups, respectively. The study author's NOAEC and LC_{50} values, based on the mean-measured concentrations, were 0.618 and 1.82 mg/L, respectively.

The positive control (potassium dichromate) yielded an LC50 value (and 95% C.I.) of 1.99 (1.91-2.08) after 24 hours of exposure. This value was within the prescribed concentration range of 0.6-2.1 mg/L of quality criteria according to OECD-Guideline for Testing of Chemicals No 202 (2004).

Table 3: Effect of Dimethyl Disulfide on Mortality of *Daphnia* sp.

TWA and (Nominal) Concentrations mg ai/L	No. of organisms	Observation Period					
		Day 0		Day 1		Day 2	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	20	0	0	0	0	0	0
0.621 (0.970)	20	0	0	0	0	1	5
1.79 (2.13)	20	0	0	8	40	9	45
3.78 (4.70)	20	0	0	12	60	20	100
8.27 (10.3)	20	0	0	17	85	20	100
17.4 (22.7)	20	0	0	12	60	20	100
45.0 (50.0)	20	0	0	20	100	20	100
NOAEC*	N/A	N/A		0.618		0.618	
LC_{50}*	N/A	N/A		272		1.82 (1.78-186)	
Positive control, if used Mortality: LC_{50} NOAEC:				LC_{50}: 1.99 (1.91-2.08)			

*The study author's toxicity values were determined using the mean measured concentrations of 0.618, 1.79, 3.69, 8.25, 17.4 and 45.1 mg ai/L.
N/A- Not Applicable

B. REPORTED SUB-LETHAL TOXICITY ENDPOINTS:

With the exception of immobility (see above), no other biological effects were reported.

C. REPORTED STATISTICS:

EC_{10} and EC_{50} values were reported to be calculated using sigmoidal dose-response regression. Calculation of the confidence intervals for the LC_{50} was reported to be done using standard procedures according to Clopper and Pearson (1937). EC_{100} values after 24 and 48 hours were deduced directly from the dose-response relationship. There was no mathematical calculation. NOAEC and LOAEC values were determined. All toxicity values were determined using the mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The 48-hour LC_{50} value (and 95% C.I.) was determined by comparing the cumulative mortality from the treatment levels to the negative control using the moving average method via Toxanal Statistical software. The NOAEC value was determined using Fisher's Exact Test via Toxstat Statistical software. As no sub-lethal effects were reported, the study author was unable to determine an EC_{50} value. All toxicity values were determined using the reviewer-calculated TWA concentrations.

LC_{50}: 1.61 mg ai/L 95% C.I.: 1.31-1.99 mg ai/L
NOAEC: 0.621 mg ai/L
EC_{50}: Not Determined 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were based on the TWA concentrations, while those of the study author were based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

In the aged solutions at 24 hours, all replicates from the two lowest treatment groups (0.970 and 2.13 mg ai/L) yielded measured values that were 21-25% higher than the TWA concentrations (0.621 and 1.79 mg ai/L). One replicate in the nominal 10.3 mg/L treatment group yielded a measured value 21% less than the TWA concentration (8.27 mg ai/L). In the aged solutions at 24 hours, all replicates in the nominal 22.7 mg/L treatment group yielded measured values that were 21-27% less than the TWA concentration (17.4 mg ai/L). In the aged solution at 48 hours, one replicate in the nominal 22.7 mg/L treatment level yielded a measured value 22% higher than the TWA concentration (17.4 mg ai/L). All other measured values throughout out the test were within 20% of the reviewer-calculated TWA concentrations.

The measured values were not always ≥70% of nominal concentrations. However, as there was no clear pattern of decline and the matrix fortifications yielded acceptable recoveries (89-102% of nominal) the reviewer used the time-weighted average concentrations.

The in-life portion of the definitive toxicity test was conducted from July 19 to July 21, 2006.

G. CONCLUSIONS:

The study is considered to be scientifically sound and is categorized Acceptable. The 48-hour NOAEC and LC_{50}

values were 0.621 and 1.61 mg ai/L, respectively.

LC_{50}: 1.61 mg ai/L 95% C.I.: 1.31-1.99 mg ai/L
NOAEC: 0.621 mg ai/L
EC_{50}: Not Determined 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
Endpoint(s) Affected: Immobility

III. REFERENCES:

AQS P 9/2 (05/1996) for daphnids clone 5 cultured in Elendt M4 medium. Bestimmung der nicht akut giftigen Wirkung von Abwasser gegenuber Daphnien uber Verdunnungsstufen (DIN 38412-L 30).

Clopper and Pearson (1934). Biometrika 26:404-413 cited in GraphPad Prism Statistics Guide 4.0.

DIN-Ritchlinie 38 412 Teil 11 (1982). Bestimmung der Wirkung von Wasserinhaltsstoffen auf Kleinkrebse.

Directive 92/69/EC L383A C.2. (1992). "Acute Toxicity to Daphnids".

ICH Harmonized Tripartite Guideline, Validation of analytical procedures: Methodology, Q2B (1996).

OECD Guideline 202 for Testing of Chemicals (adopted 13, April 2004): Daphnia sp., Acute Immobilization Test.

SANCO/3029/99 rev. 4, Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/474 (11/07/00).

U.S. EPA, Office of Prevention, Pesticides and Toxic Substances, EPA 712-C-96-114 (1996). OPPTS 850.1010, Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
45	20	20	100	9.536742E-05
17.4	20	20	100	9.536742E-05
8.27	20	20	100	9.536742E-05
3.78	20	20	100	9.536742E-05
1.79	20	9	45	41.19014
0.621	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 0.621 AND 3.78 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITSIS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC5O FOR THIS SET OF DATA IS 1.880561

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	6.572954E-02	1.618051	1.312652	1.988002

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.1686602	1	.5959495

SLOPE = 4.764876
95 PERCENT CONFIDENCE LIMITS = 2.808024 AND 6.721727

LC50 = 1.667015
95 PERCENT CONFIDENCE LIMITS = 1.279974 AND 2.077128

LC10 = .9024139
95 PERCENT CONFIDENCE LIMITS = .5079842 AND 1.195637

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	**0.621**	**20**	**1**	
2	1.79	20	9	*
3	3.78	20	20	*
4	8.27	20	20	*
5	17.4	20	20	*
6	45.0	20	20	*

APPENDIX II. REVIEWER-CALCULATED TWA CONCENTRATIONS:

TWA Concentrations (mg ai/L)										
Nominal (mg/L)	0 Hr-New	% of TWA	24 Hr-Old	% of TWA	24 Hr-New	% of TWA	48 Hr-Old	% of TWA	TWA	% of Nom.
Negative Control										
0.970										
Rep 1	0.593	96	0.774	125	0.539	87	0.616	99	0.631	
Rep 2	0.571	92	0.761	123	0.574	93	0.625	101	0.633	
Rep 3	0.453	73	0.773	125	0.559	90	0.603	97	0.597	
Rep 4	0.501	81	0.776	125	0.605	98	0.605	98	0.622	
Mean									**0.621**	64
2.13										
Rep 1	1.81	101	2.24	125	1.62	90	1.74	97	1.85	
Rep 2	1.75	98	2.17	121	1.59	89	1.73	97	1.81	
Rep 3	1.49	83	2.22	124	1.60	89	1.71	95	1.76	
Rep 4	1.59	89	2.22	124	1.52	85	1.66	93	1.75	
Mean									**1.79**	84
4.70										
Rep 1	4.00	106	3.61	96	3.73	99	4.07	108	3.85	
Rep 2	3.87	103	3.66	97	3.82	101	3.74	99	3.77	
Rep 3	3.22	85	3.81	101	3.86	102	3.95	105	3.71	
Rep 4	3.50	93	3.52	93	3.63	96	4.41	117	3.77	
Mean									**3.78**	80
10.3										
Rep 1	9.26	112	6.99	84	8.42	102	9.51	115	8.55	
Rep 2	8.90	108	7.07	85	8.82	107	9.44	114	8.56	
Rep 3	7.46	90	6.81	82	8.51	103	9.21	111	8.00	

Rep 4	7.76	94	6.53	79	8.66	105	9.04	109	8.00	
Mean									**8.27**	80
22.7										
Rep 1	17.7	102	13.3	76	19.5	112	20.2	116	17.7	
Rep 2	16.4	94	13.5	78	19.6	113	21.2	122	17.7	
Rep 3	15.7	90	13.8	79	19.8	114	20.6	118	17.5	
Rep 4	15.6	90	12.8	73	18.4	106	20.6	118	16.9	
Mean									**17.4**	77
50.0										
Rep 1	42.1	93	43.2	96	49.1	109	48.0	107	45.6	
Rep 2	44.8	99	46.9	104	46.2	103	50.4	112	47.1	
Rep 3	42.3	94	43.9	97	44.1	98	46.3	103	44.2	
Rep 4	37.7	84	42.4	94	45.9	102	47.4	105	43.4	
Mean									**45.0**	90