

## ACUTE INHALATION TOXICITY STUDY IN RATS - LIMIT TEST

**TEST METHOD NO:** P205

**STUDY NUMBER:** 13268  
Batch# 2161

**SPONSOR:** REACT, INC.  
3765 Kettle Court  
East Delafield, WI 53018

**TEST SUBSTANCE IDENTIFICATION:** ALL-Natural  
Batch# 2161

**TEST SUBSTANCE DESCRIPTION:** White to pale yellow powder

**DATE RECEIVED:** February 11, 2003

**PSL REFERENCE NO.:** 030211-5H

**DATES OF TEST:** June 6-20, 2003

### 1. PURPOSE

To provide information on health hazards likely to arise from a short-term continuous exposure (1-hour) to ALL-Natural via the inhalation route.

### 2. PROCEDURE

A group of Sprague-Dawley derived, albino rats was received from Ace Animals, Inc., Boyertown, PA. The animals were singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Rodent Chow #5012 and filtered tap water was supplied *ad libitum* by an automatic water dispensing system except during exposure.

Ten healthy rats (five males and five females) were selected for test and exposed to the test atmosphere for 1 hour. Chamber concentration and particle size distribution of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity and behavioral changes prior to exposure, approximately every 15 minutes during exposure, upon removal from the exposure chamber and at least once daily thereafter for 14 days. Body weights were recorded prior to exposure and again on Day 14 (termination).



The study was intended to be conducted at an exposure level of 200 mg/L (nominal) to satisfy the requirements of the Consumer Product Safety Commission. However, due to the limitations of the dust generation equipment, only a maximum attainable concentration of approximately 127 mg/L could be achieved.

### **3. INHALATION PROCEDURES**

#### **A. Exposure Chamber**

Rectangular whole body Plexiglas<sup>®</sup> chamber with a volume of 100 liters, with pre-chamber, operated under slight negative pressure.

#### **B. Air Supply**

Approximately 15.1 liters per minute (Lpm) of dry filtered air (JUN-AIR) was supplied to the dust generator. Compressed airflow was measured with an Omega Mass Flowmeter Model #FMA 5613. Approximately 5.1 Lpm of filtered conditioned room air was supplied as diluent air. Room airflow was measured with an Omega Mass Flowmeter, Model #FMA 5613. Chamber airflow was monitored throughout the exposure period and recorded periodically. Total airflow ranged from 20.1 to 20.3 with a mean of 20.2 Lpm.

#### **C. Ambient Conditions**

The room temperature and relative humidity ranges during exposure were 20-21°C and 60-63%, respectively. The temperature and relative humidity ranges within the exposure chamber during the test were 20-21°C and 60-68%, respectively. Room conditions were measured with a Dickson Temperature-Humidity Monitor Model TH550 and in-chamber measurements were made with a Taylor Humidity-Temperature Indicator 5502.

#### **D. Dust Generation**

The test substance was packed into the DF 183 Wright Dust Container and compressed to 500 lbs/in<sup>2</sup> using a Carver Lab Press, Model C. The container was then fitted with a DF 194 Stainless Steel cutting head and DF 191 Stainless Steel Cutting Blade, and driven by a Dayton Model 4Z538A adjustable speed motor. Compressed air was supplied to the dust generator at 25 psi.

#### **E. Nominal Chamber Concentration Measurements**

The aerosolization of the test substance and total airflow into the chamber were carefully monitored during exposure. The nominal concentration is defined as follows:

$$\text{Nominal Concentration (mg/L)} = \frac{\text{Total Test Substance Used (mg)}}{\text{Average Air Flow (LPM)} \times \text{Total Time (min)}}$$



Prior to the initiation of the study, trials were conducted to determine the proper equipment and settings needed to attain the targeted exposure concentration.

#### **F. Gravimetric Chamber Concentration Measurements**

Gravimetric samples were withdrawn on two occasions from the breathing zone of the animals. Samples were collected using 25 mm glass fiber filters (GF/B Whatman) in a filter holder attached by 1/4 inch tygon tubing to an Reliance Electric vacuum pump Model #G557X. Filter papers were weighed before and after collection to determine the mass collected. This value was divided by the total volume of air sampled to determine the chamber concentration. The collections were carried out for one minute at airflows of 4 Lpm.

#### **G. Particle Size Distribution**

An eight-stage Andersen cascade impactor was used to assess the particle size distribution of the test atmosphere withdrawn from the breathing zone of the animals. The filter paper collection stages were weighed before and after sampling to determine the mass collected at each stage. The aerodynamic mass median diameter and geometric standard deviation were determined graphically using two-cycle logarithmic probit axes.

#### **H. Exposure Period**

The animals were exposed to the test atmosphere for 1 hour and 23 minutes. The exposure period was extended beyond 1 hour to allow the chamber to reach equilibrium ( $T_{99}$ ). The times for 90 and 99% equilibration of the atmosphere were 11.4 and 22.8 minutes, respectively. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 23 minutes with clean air. At the end of this period the animals were removed from the chamber. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.

### **4. RESULTS**

Nominal and gravimetric chamber concentrations are shown in Table 1. Particle size sampling results are presented in Table 2. Individual body weights are presented in Table 3. Cage-side observations are shown in Table 4.

All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. The nominal chamber concentration was 126.98 mg/L. The mass median aerodynamic diameter was estimated to be 5.0 microns based on the particle size distribution as measured with an Anderson Cascade Impactor.

In-chamber animal observations included hunched posture and hypoactivity. All animals recovered from these symptoms upon removal from the exposure chamber and appeared active and healthy over the 14-day observation period.

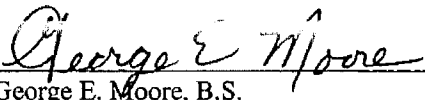
### **5. CONCLUSION**

The acute inhalation  $LC_{50}$  of ALL-Natural is greater than 126.98 mg/L (nominal, maximum attainable).

**SIGNATURES**

ALL-Natural

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

  
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George E. Moore, B.S.  
Study Director  
Product Safety Labs

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Date 7/28/03

**TABLE 1: NOMINAL CHAMBER CONCENTRATION**

Target Exposure Level (mg/L)	Total Test Substance Used (g)	Average Total Airflow (Lpm)	Total Time of Exposure (min)	Nominal Concentration <sup>1</sup> (mg/L)
200	212.9	20.2	83	126.98

**GRAVIMETRIC CHAMBER CONCENTRATIONS**

Sample Number	Time of Sampling (hour)	Mass Collected (mg)	Airflow Sampled (Lpm)	Collection Time (min)	Chamber Concentration (mg/L)
1	0.5	28.3	4	1	7.08
2	1	31.4	4	1	7.85
Average ± Standard Deviation					7.47 ± 0.54

$$^1 \text{ Nominal Concentration} = \frac{\text{Total Test Substance Used (mg)}}{\text{Average Airflow (LPM)} \times \text{Total Time (min)}}$$

**TABLE 2: PARTICLE SIZE DISTRIBUTION**

Stage	Effective Cut off Diameter ( $\mu\text{m}$ )	% of Total Particles Captured (by weight)	Cumulative (%) <sup>1</sup>
0	9.0	15.5	84.5
1	5.8	25.1	59.3
2	4.7	15.2	44.0
3	3.3	19.2	24.9
4	2.1	14.5	10.4
5	1.1	6.2	4.1
6	0.7	2.8	1.3
7	0.4	1.3	0.0
F	0.0	0.0	0.0

**SUMMARY OF PARTICLE SIZE DISTRIBUTION**

Time of Sample (hour)	Collection Time (minutes)	Mass Median Aerodynamics Diameter ( $\mu\text{m}$ )	Geometric Standard Deviation
0.75	1	5.0	2.29

<sup>1</sup> Percent of particles smaller than corresponding effective cutoff diameter.

**TABLE 3: INDIVIDUAL BODY WEIGHTS**

Animal No.	Sex	Body Weight (g)	
		Initial	Day 14
9508	M	320	397
9509	M	297	412
9510	M	294	386
9511	M	322	441
9512	M	335	424
9513	F	220	247
9514	F	199	236
9515	F	211	234
9516	F	202	241
9517	F	228	256



TABLE 4: INDIVIDUAL CAGE-SIDE OBSERVATIONS

<u>Animal Number</u>	<u>Findings</u>	<u>Day of Occurrence</u>
<u>MALES</u>		
9508-9512	Active and healthy	CR <sup>1</sup> -14
<u>FEMALES</u>		
9513-9517	Active and healthy	CR-14

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<sup>1</sup> CR - removal from exposure chamber