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*Industrial BIO-TEST Laboratories, Inc.*

1810 FRONTAGE ROAD  
NORTHBROOK, ILLINOIS 60062

October 14, 1975

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(b) (4)

Re: (b) (4) - Acute Dust Inhalation Toxicity Studies  
and Acute Vapor Inhalation Toxicity Studies with

(b) (4)

We are submitting herewith our laboratory reports prepared in  
connection with the above study.

Very truly yours,

J. C. Calandra  
President

JCC:bp

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*Industrial BIO-TEST Laboratories, Inc.*  
1810 FRONTAGE ROAD  
NORTHBROOK, ILLINOIS 60062

REPORT TO

(b) (4)

ACUTE DUST INHALATION TOXICITY STUDY WITH  
(b) (4) (b) (4)  
IN ALBINO RATS

OCTOBER 14, 1975

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ACUTE DUST INHALATION TOXICITY STUDY IN RATS

Test Material: (b) (4) (b) (4)  
Form Administered: Dust  
Acute LC<sub>50</sub>: > 960 mg/m<sup>3</sup> air

Strain: Charles River Rats  
Exposure Time: 4 hours  
Observation Period: 14 days

(b) (4)

Generation of Material Exposure:

The dust was suspended by passing clean, dry air (-40°C dewpoint) through a ferris wheel dust mechanism. The resulting air and dust mixture was then introduced into the exposure chamber.

<u>Chamber Conditions</u>		<u>Atmospheric</u>		<u>Air Flow</u>
<u>Group No.</u>	<u>Size (liters)</u>	<u>Pressure (inches Hg)</u>	<u>Temperature (°C)</u>	<u>(l/min)</u>
I	80	30.20	25	50

  

<u>Results</u>	<u>Total Number of Animals</u>	<u>Analytical Concentration</u>	<u>Mortality Male-Female</u>	<u>Weight Gain Male-Female (grams)</u>
<u>Group No.</u>	<u>Male/Female</u>			
I	5/5	960 mg/m <sup>3</sup> air	0/5 - 0/5	86-42

Remarks

Reactions are presented in Table I.

The average 2-week body weight gains were within the normal limits. Necropsy, performed on all rats at the end of the observation period, did not reveal any gross pathologic alterations.

Particle size distribution data are present in Table II.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Prepared by: Terry W. Myers  
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John W. Goode, Ph.D.  
Manager  
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TABLE I

TEST MATERIAL: (b) (4) (b) (4)

Acute Dust Toxicity Study - Rats

Reactions

Reaction	Number of Animals Affected	Time of Onset After Start of Exposure (min)	Duration (min)
Lacrimation	1	10	230
Salivation	1	10	230
Lacrimation	9	35	205
Salivation	9	35	205

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Particle Size Distribution

A sample of airborne dust was collected from the test atmosphere for the purpose of conducting a microscopic determination of particle size distribution. Particles were counted with respect to 4 size ranges, viz. 5 microns or smaller, 6 to 10 microns, 11 to 25 microns and larger than 25 microns. Particles less than 10 microns are generally considered to be respirable. The smallest particle which can be detected by the light-field technique employed is approximately 1 micron. The smallest and largest particles observed were also recorded.

TABLE I

TEST MATERIAL: (b) (4) (b) (4)

Particle Size Distribution Data

Particle Size Range (microns)	Number of Particles Counted	Percent of Total Counted
1-5	0	0
6-10	0	0
11-25	53	13
> 25	340	87

The total number of particles counted was 393.

The smallest and largest particles observed were 11 and 125 microns, respectively.

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## PROCEDURE FOR ACUTE DUST INHALATION TOXICITY STUDY

Young adult albino rats were employed as test animals. The rats were selected after having been under observation for at least 5 days to insure their general health and suitability for testing. The animals were housed individually in stock cages and permitted a standard laboratory diet\* plus water ad libitum, except during inhalation exposure.

During the exposure period, observations were made with respect to incidence of mortality and reactions displayed. At the end of the exposure period, the rats were returned to their cages for observation.

A body weight was determined for each animal prior to inhalation exposure and for each surviving animal at the end of the observation period. The data were recorded as an index to growth.

Necropsy examinations were scheduled to be conducted upon all animals which might succumb during the test period and upon those sacrificed at the end of the observation period.

Test animals were exposed in a specially constructed inhalation chamber. The chamber was designed so that the animals could be introduced into the test atmosphere after the desired dust concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur.

Dust was suspended with a specially designed dust feeder capable of producing high concentrations over a long period of time. The test material powder was passed through a high-velocity stream of clean, dry air (-40°C dewpoint). The air-jet velocity was adjusted to obtain the desired concentration of suspended dust. The test atmosphere was then introduced into the exposure chamber at the top center, dispersed by a baffle plate and exhausted at the bottom of the chamber. Air flow rate through the system was measured with a rotameter connected in the air supply line upstream of dust contamination. The rotameter was calibrated with a wet-test meter after the exposure was completed.

The concentration of test material dust present in the exposure chamber was determined by sampling the test atmosphere in the breathing zone of the animals being exposed. The total weight of dust collected on a glass fiber filter\*\* was divided by the total volume of air drawn through the filter during the sampling period. Air flow rate for sampling was regulated by a calibrated limiting orifice. The average analytical concentration of airborne dust was obtained by repeated air sampling. Whenever possible, the LC<sub>50</sub> was calculated using the method of Litchfield and Wilcoxon\*\*\*.

\* Wayne LAB-BLOX for Rats, Allied Mills, Inc., Chicago, Illinois.

\*\* Gelman Instrument Co., Ann Arbor, Michigan; Type A filter, advertised as 99.7 percent efficient, 0.3 micron DOP aerosol test.

\*\*\* Litchfield, J. T., Jr. and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther. 96, 99 (1949).

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