PRODUCT

Ferro Niobium (FeNb)

STUDY TITLE

Acute Inhalation Toxicity Study in Rats

DATA REQUIREMENTS

U.S. EPA Health Effects Test Guidelines, OPPTS 870.1300

OECD Guidelines for the Testing of Chemicals, Test No. 403

AUTHOR

S. Dana Oley, B.A.

STUDY COMPLETED ON

November 10, 2009

PERFORMING LABORATORY

Eurofins | Product Safety Laboratories

LABORATORY STUDY NUMBER

27973

eurofins|Product Safety Laboratories
2394 US Highway 130
Dayton, NJ 08810
USA

T | 732-438-5100
F | 732-355-3275
psl@productsafetylabs.com
www.productsafetylabs.com

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: **CBMM EUROPE BV**

Company Agent:

_____	_____
Name	Title
_____	_____
Signature	Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Ferro Niobium (FeNb)

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), 1989 and OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM (98)17, OECD, Paris, 1998. Specific information related to the characterization of the test substance as received and tested is the responsibility of the study Sponsor (see Test Substance section).

Study Director: S. Dana Oley

S. Dana Oley, B.A.
Eurofins | Product Safety Laboratories

11/10/2009
Date

Submitter: _____
Signature

_____
Date

Sponsor: _____
Signature

_____
Date

QUALITY ASSURANCE STATEMENT

The Eurofins | Product Safety Laboratories' Quality Assurance Unit has reviewed this final study report to assure the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	Jan 24, 2007[1]; Oct 11, 2009	Jan 24, 2007; Oct 14, 2009
In-process inspection: *Day 4 in-life observations*	Aug 10, 2009	Oct 14, 2009
Raw data audit	Oct 11, 2009	Oct 14, 2009
Draft report review	Oct 11, 2009	Oct 14, 2009

Stephanie Mullin
Quality Assurance Auditor
Eurofins | Product Safety Laboratories

Nov. 9, 2009
Date

[1] EPSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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ACUTE INHALATION TOXICITY STUDY IN RATS

PROTOCOL NO.:	P330
AGENCY:	EPA (FIFRA) and OECD
STUDY NUMBER:	27973
SPONSOR:	CBMM EUROPE BV WTC H-Tower, Zuidplein 96 1077 XV, Amsterdam
TEST SUBSTANCE IDENTIFICATION:	Ferro Niobium (FeNb) Lot #AD/4204
DATE RECEIVED:	July 28, 2009
EPSL REFERENCE NO.:	090728-3D
STUDY INITIATION DATE:	July 30, 2009
DATES OF TEST:	August 5 – 20, 2009
NOTEBOOK NO.:	09-168: pages 138-178

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Ferro Niobium (FeNb) by the inhalation route.

2. SUMMARY

An acute inhalation toxicity test was conducted with rats to determine the potential for Ferro Niobium (FeNb) to produce toxicity via the inhalation (nose-only exposure) route. Under the conditions of this study, the single exposure acute inhalation LC_{50} of the test substance is greater than 2.07 mg/L in male and female rats.

After establishing the desired generation procedures during pre-test trials, ten healthy rats (5/sex) were exposed to the test atmosphere for 4 hours. Chamber concentration and particle size distributions of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days following exposure. Body weights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. The gravimetric chamber concentration was 2.07 mg/L. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 3.95 μm.

Following exposure and over the entire 14-day observation period, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The test substance, identified as Ferro Niobium (FeNb), Lot #AD/4204, was received on July 28, 2009 and was further identified with EPSL Reference Number 090728-3D. The test substance was stored at room temperature. The test substance was aerosolized as received. Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the Sponsor.

The following information related to the characterization of the test substance was provided by the Sponsor:

Composition: Nb > 63%
Fe ~ 30%

Physical Description: Silver gray metallic solid

pH: Not Available

Solubility: Not Provided.

Stability: Test substance was expected to be stable for the duration of testing.

Expiration Date: Not Applicable.

B. Animals

3.B.1 Number of Animals: 10

3.B.2 Sex: 5 Males and 5 Females. Females assigned to test were nulliparous and non-pregnant.

3.B.3 Species/Strain: Rat/Sprague-Dawley derived, albino.

3.B.4 Age/Body weight: Young adult (8-9 weeks)/males 245-279 grams and females 196-218 grams at experimental start.

3.B.5 Source: Received from Ace Animals, Inc., Boyertown, PA on July 28, 2009.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel caging with mesh floors which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature and Relative Humidity Ranges: 19-24°C and 59-80%, respectively. The humidity was above the targeted upper limit for 9 days during the study. A portable dehumidifier was used to lower the humidity levels during this time.

4.A.3 Photoperiod: 12-hour light/dark cycle

4.A.4 Acclimation Period: 9 days

4.A.5 Food: Purina Rodent Chow #5012

4.A.6 Water: Tap water was supplied *ad libitum* by an automatic water dispensing system except during exposure.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted regularly and the records are kept on file at Eurofins | Product Safety Laboratories.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: A number was allocated to each rat on receipt and a stainless steel ear tag bearing this number was attached to the rat. This number, together with a sequential animal number assigned to study 27973, constituted unique identification.

5. PROCEDURE

A. Pre-Test Trials

Prior to initiation of the full inhalation study, pre-test trials were conducted to establish generation procedures to achieve, to the extent possible, the desired chamber concentration (2.0 mg/L) and desired particle size distribution (mass median aerodynamic diameter between 1 and 4 μm). In these trials, the following adjustments were made in an attempt to achieve these objectives:

Air Pressure:	constant
Compressed Generator Airflow:	constant
Compressed Mixing Airflow:	constant
Total Airflow:	constant
Motor Setting:	varied
Dust Generating System:	constant
Cutting Head:	constant

Cutting Blade: constant
Packing Pressure: constant

The procedures and aerosolization equipment used in the full test were based on the results of pre-test trial number 2. This provided a chamber concentration of 2.04 mg/L and a mass median aerodynamic diameter of 3.7 µm.

B. Inhalation Procedures

The exposure chamber, air supply and equipment used to measure particle size distribution, airflow and chamber concentration were the same as used during the pre-test trials and are described below.

5.B.1 Nose-Only Exposure Chamber: A nose-only inhalation chamber with an internal volume of approximately 6.7 liters (Mini-Nose Only Inhalation Chamber, ADG Developments LTD) was used for exposure. Animals were individually housed in polycarbonate holding tubes which seal to the chamber with an "O" ring during exposure. The base unit terminates the chamber with a 0.5-inch diameter tube for discharged air.

5.B.2 Air Supply: Approximately 28.4 liters per minute (Lpm) of filtered air was supplied by an air compressor (JUN-AIR, Model #6-15) to the dust generator. An additional 3.3 Lpm of compressed mixing air, supplied using air from a compressed air tank (Airgas), which was introduced into the chamber to help uniformly distribute the test atmosphere by creating a vortex at the chamber inlet. Compressed airflow was measured with a Mass Flowmeter (Omega, Model #FMA-5613). Chamber airflow was monitored throughout the exposure period and recorded periodically. Total airflow ranged from 31.5 to 31.9 with a mean of 31.7 Lpm. Based on the volume of the inhalation chamber, this airflow provided approximately 284 air changes per hour during the study.

5.B.3 Ambient Conditions: The exposure tube temperature and relative humidity ranges during exposure were 21-23°C and 63-70%, respectively. The room temperature and relative humidity ranges during exposure were 20-21°C and 64-67%, respectively. In-chamber measurements were made with a Humidity-Temperature Indicator (Taylor, Model #5502) and room conditions were measured with a Temperature-Humidity Monitor (Dickson, Model #TH550). Temperature and relative humidity values were recorded every 15 minutes for the first hour of exposure and every 30 minutes thereafter.

5.B.4 Dust Generation: The test substance was aerosolized using a modified Wright Dust Generator driven by a variable speed motor (Dayton, Model #4Z538A) D.C. speed control with 0-100 potentiometer. The test substance was packed into the dust container (Wright, Model DF183) and compressed to 2,500 lbs/in^2 using a lab press (Carver, Model C). The container was then fitted with a stainless steel cutting head (Model DF194SS) and cutting blade (Model DF191SS). Compressed air was supplied to the dust generator at 30 psi. The aerosolized dust was then fed directly into the chamber through the dust outlet assembly.

5.B.5 Chamber Concentration Measurements: Gravimetric samples were withdrawn at 6 intervals 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 ¼ inch Tygon tubing to a vacuum pump (Reliance Electric, 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 2 minutes at airflows of 4 Lpm. Sample airflows were measured using a Mass Flowmeter (Omega, Model #FMA-5610).

5.B.6 Particle Size Distribution: An eight-stage Andersen cascade impactor was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The filter paper collection stages were weighed before and after sampling to determine the mass collected upon each stage. The aerodynamic mass median diameter and geometric standard deviation were determined graphically using two-cycle logarithmic probit axes.

5.B.7 Exposure Period: The animals were exposed to the test atmosphere for 4 hours and 1 minute. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T_{99}). The times for 90 and 99% equilibration of the chamber atmosphere were 0.5 and 1.0 minute, respectively. At the end of the exposure period, the generation was terminated and the chamber was operated for a further 15 minutes with clean air. At the end of this period the animals were removed from the exposure tube. Prior to being returned to their cages, excess test substance was removed from the fur of each animal.

C. Selection of Animals

On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naive rats (five males and five females; not previously tested) were selected for test.

D. Body Weights

Individual body weights of the animals were recorded prior to test substance exposure (initial) and again on Days 7 and 14 (termination).

E. Cage-Side Observations

All animals were observed for mortality during the exposure period. The animals were examined for signs of gross toxicity, and behavioral changes upon removal from the exposure chamber and at least once daily thereafter for up to 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma.

F. Necropsy

All rats were euthanized via CO_2 inhalation on Day 14. Gross necropsies were performed on all animals. Tissues and organs of the thoracic and abdominal cavities were examined.

6. STUDY CONDUCT

This study was conducted at Eurofins | Product Safety Laboratories, 2394 US Highway 130, Dayton, New Jersey 08810. The primary scientist for this study was Jasbir Bawa, B.S. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 40 CFR 160: U.S. EPA GLP Standards: Pesticide Programs (FIFRA), 1989

- OECD Principles of GLP (as revised in 1997) published in ENV/MC/CHEM (98)17, OECD, Paris, 1998

and based on the following testing guidelines:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.1300
- OECD Guidelines for Testing of Chemicals, Test No. 403

7. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Eurofins | Product Safety Laboratories Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

8. DEVIATIONS FROM FINAL PROTOCOL

None

9. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Eurofins | Product Safety Laboratories, is maintained in the Eurofins | Product Safety Laboratories Archives. EPSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or may request continued archiving by EPSL.

10. RESULTS

Details of all pretest exposure trials are described in Tables 1 through 3. A summary of test exposure information is presented in Tables 4 through 6. Individual body weights, and cage-side and necropsy observations are presented in Tables 7 through 9, respectively.

All animals survived exposure to the test atmosphere and gained body weight over the 14-day observation period. The gravimetric and nominal chamber concentrations were 2.07 mg/L and 24.56 mg/L, respectively. The mass median aerodynamic diameter was estimated to be 3.95 µm based on the particle size distribution as measured with an Andersen Cascade Impactor.

Following exposure and over the entire 14-day observation period, all animals appeared active and healthy. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

11. CONCLUSION

Under the conditions of this study, the single exposure acute inhalation LC_{50} of Ferro Niobium (FeNb) is greater than 2.07 mg/L in male and female rats.

SIGNATURE

Ferro Niobium (FeNb)

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.

S. Dana Oley

S. Dana Oley, B.A.
Study Director
Eurofins | Product Safety Laboratories

11/10/2009
Date

TABLE 1: PREPARATION AND GENERATION SYSTEM FOR PRE-TEST TRIALS

1. Dust Generator:	Wright (Modified)
2. Drive Motor:	Dayton, Model #4Z538A D.C. speed control with 0-100 potentiometer
3. Air Supply:	Air Compressor (JUN-AIR, Model #6-15) Compressed air (Airgas)
4. Dust Container:	Model DF183
5. Cutting Head/Blade:	Stainless steel head, Model DF194SS Stainless steel blade, Model DF191SS
6. Chamber:	6.7 liter (Mini Nose-Only Inhalation Chamber, ADG Developments LTD)
7. Diluent Airflow Measurements:	Mass Flowmeter (Omega, Model #FMA-5613)
8. Lab Press	Carver, Model C

TABLE 2: PRE-TEST EXPOSURE TRIALS[1]

Trial No.	Compressed Air Pressure (psi)	Compressed Air Volume (Lpm)	Compressed Mixing Air (Lpm)	Total Air Volume (Lpm)	Dust Generator Motor Setting	Chamber Conc. (mg/L)	Particle Size Sampled
1	30	28.5	3.3	31.8	10.0	1.59	No
2	30	28.4	3.3	31.7	10.25	2.04	Yes

[1] Test substance used as received.

TABLE 3: SUMMARY OF PRE-TEST EXPOSURE TRIAL[1]

Trial No.	Chamber Concentration (mg/L)	Mass Median Aerodynamic Diameter[2] (µm)
2	2.04	3.7

[1] See Tables 1 and 2 for details of generation system applicable to the trial.

[2] This figure is an estimation based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor.

TABLE 4: GRAVIMETRIC CHAMBER CONCENTRATIONS

Sample Number	Time of Sample (hour)	Mass Collected (mg)	Airflow Sampled (Lpm)	Collection Time (min)	Chamber Concentration (mg/L)
1	0.5	13.5	4	2	1.69
2	1	16.8	4	2	2.10
3	2	14.6	4	2	1.83
4	2.5	18.1	4	2	2.26
5	3.5	18.4	4	2	2.30
6	3.75	18.0	4	2	2.25
				Average ± Standard Deviation	2.07 ± 0.25

TABLE 5: PARTICLE SIZE DISTRIBUTION

Stage	Effective Cutoff Diameter (µm)	% of Total Particles Captured (by weight)	Cumulative (%)[1]
Sample 1			
0	9.0	14.4	85.6
1	5.8	17.1	68.4
2	4.7	11.6	56.9
3	3.3	20.4	36.4
4	2.1	16.9	19.6
5	1.1	11.3	8.2
6	0.7	5.6	2.7
7	0.4	1.6	1.1
F	0.0	1.1	0.0
Sample 2			
0	9.0	15.8	84.2
1	5.8	18.1	66.1
2	4.7	10.1	56.0
3	3.3	18.8	37.2
4	2.1	17.8	19.3
5	1.1	10.3	9.0
6	0.7	6.3	2.8
7	0.4	2.0	0.8
F	0.0	0.8	0.0

[1] Percent of particles smaller than corresponding effective cutoff diameter.

TABLE 6: SUMMARY OF PARTICLE SIZE DISTRIBUTION

Sample No.	Time of Sample (hours)	Collection Time (minutes)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
1	1.5	2	3.9	2.33
2	3	2	4.0	2.38

TABLE 7: INDIVIDUAL BODY WEIGHTS

Animal No.	Sex	Body Weight (g)		
		Initial	Day 7	Day 14
3301	M	245	292	340
3302	M	265	313	368
3303	M	255	296	351
3304	M	275	330	379
3305	M	279	333	378
3306	F	196	208	234
3307	F	207	208	241
3308	F	203	217	250
3309	F	218	219	249
3310	F	198	208	240

TABLE 8: INDIVIDUAL CAGE-SIDE OBSERVATIONS

Animal Number	Findings	Day of Occurrence
MALES		
3301 – 3305	Active and healthy	CR[1]-14
FEMALES		
3306 – 3310	Active and healthy	CR-14

[1] CR - removal from exposure tube

TABLE 9: INDIVIDUAL NECROPSY OBSERVATIONS

Animal Number	Tissue	Findings
MALES		
3301 – 3305	All tissues and organs	No gross abnormalities
FEMALES		
3306 – 3310	All tissues and organs	No gross abnormalities