STUDY TITLE

Ammonium Niobium Oxalate:
Acute Inhalation Toxicity in Rats

DATA REQUIREMENT

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

AUTHOR

Daniel Merrill, BS, MBA

STUDY COMPLETED ON

Final Report: September 26, 2014

PERFORMING LABORATORY

Product Safety Labs

LABORATORY STUDY NUMBER

38154

BSL PROJECT NUMBER

13-6511

Product Safety Labs
2394 US Highway 130
Dayton, NJ 08810
USA

T | 732-438-5100
F | 732-230-4209
psl@productsafetylabs.com
www.productsafetylabs.com

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Ammonium Niobium Oxalate

This study meets the requirements of 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:_____ Date: 9/26/14

Name of Signer: Daniel Merrill, BS, MBA

Name of Company: Product Safety Labs

Sponsor:_____ Date:_____

Name of Signer: _____

Name of Company: CBMM Europe BV

Submitter:_____ Date:_____

Name of Signer: _____

Name of Company: CBMM Europe BV

QUALITY ASSURANCE STATEMENT

The Product Safety Labs' 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	Oct 1, 2013[1]; July 28, 2014	Oct 1, 2013; July 28, 2014
In-process inspection: *Day 13 in-life observations*	Mar 18, 2014	July 28, 2014
Raw data audit	July 28, 2014	July 28, 2014
Draft report review	July 28 and 31, 2014	July 28 and 31, 2014

Final report reviewed by:

Annamarie LaPorte, RQAP-GLP
Quality Assurance Auditor
Product Safety Labs

Sept 26, 2014
Date

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

TABLE OF CONTENTS

AMMONIUM NIOBIUM OXALATE: ACUTE INHALATION TOXICITY IN RATS

PROTOCOL NO.:	P330
AGENCY:	OECD
STUDY NUMBER:	38154
SPONSOR:	CBMM Europe BV WTC H-Tower Zuidplein 96 Amsterdam, The Netherlands 1077 XV
TEST SUBSTANCE IDENTIFICATION:	Ammonium Niobium Oxalate
BATCH NO.:	AD/4663
IUPAC NAME:	Reaction mass of ammonium oxobis(ethanedioato) bisaquo niobate(V) hydrates and ammonium hydrogen ethanedioate ethanedioic acid dihydrate
PHYSICAL STATE AT RT:	Powder
COLOR:	White
PURITY:	≥ 96%
EXPIRY DATE:	March 25, 2015
DATE RECEIVED/CONDITION AT RECEIPT:	November 25, 2013/received in good condition
STORAGE:	At room temperature
SAFETY PRECAUTIONS:	Routine laboratory hygienic procedures
PSL REFERENCE NO.:	131125-5R
STUDY INITIATION DATE:	February 10, 2014
DATES OF TEST:	February 20 – March 19, 2014
NOTEBOOK NO.:	14-38154: pages 1-67

1. PURPOSE

To provide information on health hazards likely to arise from a short-term exposure to Ammonium Niobium Oxalate by the inhalation route.

2. SUMMARY

An acute inhalation toxicity test was conducted with rats to determine the potential for Ammonium Niobium Oxalate to produce toxicity from a single exposure via the inhalation (nose-only exposure) route. Under the conditions of this study, the acute inhalation LC_{50} of the test substance is greater than 5.10 mg/L in both sexes.

After establishing the desired generation procedures during the 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 or until death occurred. Body weights were recorded prior to exposure (initial) and again on Days 1, 3, 7, and 14 (terminal) or after death. Necropsies were performed on all animals.

The gravimetric chamber concentration was 5.10 mg/L. The average mass median aerodynamic diameter was estimated to be 2.93 µm based on graphic analysis of the particle size distribution as measured with a 1 ACFM Andersen Ambient Particle Sizing Sampler with an average geometric standard deviation of 2.42.

One male and three females died within seven days of exposure to the test atmosphere. Prior to death, the animals were hypoactive and exhibited abnormal respiration and/or ano-genital staining. Following exposure, all surviving animals were hypoactive and exhibited abnormal respiration. In addition, several survivors exhibited ano-genital staining, ocular discharge, facial staining, and/or nasal discharge. Although all animals lost weight through Day 3, the animals gained body weight during Days 7 to 14. However, two of the survivors did not surpass their initial body weights by the end of the 14-day observation period. Gross necropsy of the decedents revealed discoloration of the lungs, distention of the stomach, discoloration of the liver and/or distention of the intestines. No gross abnormalities were noted for any of the surviving animals when necropsied at the conclusion of the 14-day observation period.

3. MATERIALS

A. Test Substance

The test substance, identified as Ammonium Niobium Oxalate, Batch #: AD/4663, was received on November 25, 2013, and was further identified with PSL Reference Number 131125-5R. The test substance was stored at room temperature. Prior to aerosolization, the test substance was ground in a coffee mill (Cuisinart, Model #DCG-20N). Documentation of the methods of synthesis, fabrication, or derivation of the test substance is retained by the sponsor. At the request of the sponsor, a reserve sample of the test substance was retained for possible future qualitative analysis.

The following information related to the characterization of the multi-constituent test substance was provided by the sponsor:

Chemical name: Reaction mass of ammonium diaqua[bis(oxalate)]oxoniobate(1-) hydrate and ammonium hydrogen oxalate oxalic acid (1:1:1) dehydrate

Purity: ≥ 96%

Composition: Ammonium oxobis(ethanedioato) bisniobate(V) hydrates – ca. 70%
Ammonium hydrogen ethanedioate ethanedioic acid dehydrate – ca. 27%
Free water – ca. 2.5%
Organic and inorganic impurities – ca. 0.5%

Physical Description: White powder

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

Expiration Date: 25.03.2015

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 weeks)/males 242-267 grams and females 170-199 grams at experimental start.

3.B.5 Source: Received from Harlan Laboratories, Inc. on February 19, 2014.

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were singly housed in suspended stainless steel perforated bottom caging which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals* (Natl. Res. Council, 2011). Enrichment (e.g., toy) was placed in each cage. 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: 20-23°C and 45-55%, respectively.

4.A.3 Animal room air changes/hour: 12. Airflow measurements are evaluated regularly and the records are kept on file at Product Safety Labs.

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

4.A.5 Acclimation period: 14 days

4.A.6 Food: Harlan Teklad Global 16% Protein Rodent Diet® #2016. The diet was available *ad libitum*, except during the exposure.

4.A.7 Water: Filtered tap water was supplied *ad libitum,* except during exposure.

4.A.8 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 Product Safety Labs.

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 38154, 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 (5.0 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:	varied
Compressed Generator Airflow:	varied
Compressed Mixing Airflow:	constant
Total Airflow:	varied
Motor Setting:	varied
Helix Size:	varied
Dust Generating System:	varied
Packing Pressure:	varied
Material Preparation:	varied
Vacuum Pump:	constant
Concentration Sampling Time:	constant

An initial attempt to aerosolize the ground test substance using a Jet-Mill was ineffective utilizing ¼, ½, and ⅜" helices. An attempt using the Modified Wright Dust Feeder failed due to test substance not packing properly in the aerosolization cup using the lab press (Carver, Model C). Next, an attempt to liquefy the test substance for aerosolization also failed. Additionally, attempts to solubilize the test substance as a 25% and 50% (w/w) dilution in distilled water were also unsuccessful as a uniform mixture could not be attained.

The procedures and aerosolization equipment used in the full test were based on the results of pre-test trial number 5. This provided a chamber concentration of 5.28 mg/L and a mass median aerodynamic diameter of 2.98 µm. The test substance used in trial numbers 1-5 (as well as the full test) was ground prior to aerosolization. The material feeder was maintained with a limited amount of test substance to ensure that there was consistent supply of material into the jet mill. During the trials, the exposure system and the aerosolization equipment were electrically grounded.

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 appropriate pre-test trial and are described below.

5.B.1 Nose-only exposure chamber: A nose-only inhalation chamber with an internal volume of approximately 28 liters (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. During the exposure, the exposure system and the aerosolization equipment were electrically grounded.

5.B.2 Air supply: Approximately 60.0 liters per minute (Lpm) of filtered generator air was supplied by an air compressor (Powerex Model: SES050822), measured with a Mass Flow Controller (Omega, Model #FMA-5541) to the dust generator. An additional 10.0 Lpm of filtered mixing air, from the same air compressor, measured with a Mass Flow Controller (Omega, Model # FMA-5527), was introduced into the chamber to help uniformly distribute the test atmosphere by creating a vortex at the chamber inlet. Chamber airflow was monitored throughout the exposure period and recorded periodically. Total airflow was 70.0 Lpm. Based on the volume of the inhalation chamber, this airflow provided approximately 150 air changes per hour during the study. The exposure was conducted under slightly negative pressure.

5.B.3 Ambient conditions: The exposure chamber temperature and relative humidity range during exposure were 20°C and 22-24%, respectively. The room temperature and relative humidity ranges during exposure were 20-21°C and 23-26%, respectively. A humidifier was used in the room during exposure. The measurements inside the exposure chamber and room conditions were measured with a Temperature-Humidity Monitor (Fisher Scientific, Model #11-661-18). Temperature and relative humidity values were recorded every 15 minutes for the first hour of exposure approximately and every 15 or 30 minutes thereafter.

5.B.4 Dust generation: The test substance was aerosolized using a Jet-Mill (Fluid Energy Jet-Mill Serial #J2724E). The test substance was delivered from the hopper using a variable speed motor (Accurate Series 100 Dry Material Feeder) into the Jet-Mill. The material feeder was maintained with a limited amount of test substance to ensure that there was consistent supply of material into the jet mill. Compressed generator/mixing air was supplied to the dust generator at 60/30 psi, respectively. 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 37 mm glass fiber filters (Whatman™ GF/B) in a filter holder attached by ¼ inch Tygon® tubing to a vacuum pump (GAST, Model #1531-107B-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 1 minute at airflows of 4.0 Lpm. Sample airflows were measured using a Mass Flow Controller (Aalborg, Model #GFC-17).

5.B.6 Particle size distribution: An eight-stage 1 ACFM Andersen Ambient Particle Sizing Sampler 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 mass median aerodynamic

diameter (MMAD) and geometric standard deviation (GSD) 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 2 minutes. 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 0.92 and 1.84 minutes, 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. Following removal from the chamber and prior to being returned to their cages, excess test substance was removed from the fur of each animal by rinsing with tap water and clean paper towels.

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 1, 3, 7, and 14 (terminal) or after death.

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 tube and at least once daily thereafter for 14 days or until death occurred. 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

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

6. STATISTICAL ANALYSIS

Statistical analysis was limited to the calculation of the mean and standard deviation.

7. STUDY CONDUCT

This study was conducted at Product Safety Labs' (PSL) test facility at 2394 US Highway 130, Dayton, New Jersey 08810. The Study Director for this study was Daniel Merrill, BS, MBA. The primary scientist for this study was Mark Schooley, with contributions from Cynthia Bodnar, Matthew Notta, BS, and Maryann Zakrzewski. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

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

and based on the following testing guideline:

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

8. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Labs 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.

9. AMENDMENT TO THE PROTOCOL

The Records to be Maintained section of the Protocol was changed as indicated below:

The original signed final report will be sent to the sponsor. A copy of the signed report, together with the original raw data, documentation, records and the original protocol, associated amendments and/or deviations if applicable, generated at PSL will be stored in the PSL archives. At the request of the Sponsor, PSL will maintain these records for a period of at least fifteen years, unless otherwise requested. After this time, the sponsor of the study will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL. Any copies of the raw data, protocol and final report, along with all study correspondence can be destroyed after this time.

10. DEVIATIONS FROM THE PROTOCOL

None.

11. FINAL REPORT AND RECORDS TO BE MAINTAINED

Information on care of the test system, equipment maintenance and calibration, storage, usage, and disposition of the test substance, and all other records that would demonstrate adherence to the protocol will be maintained. Facility records which are not specific to the subject study will be maintained by the testing facility and archived according to PSL SOP.

The original signed final report will be sent to the sponsor. A copy of the signed report, together with the original raw data, documentation, records and the original protocol, associated amendments and/or deviations if applicable, generated at PSL will be stored in the PSL Archives. At the request of the sponsor, PSL will maintain these records for a period of at least fifteen years, unless otherwise requested. After this time, the sponsor of the study will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL. Any copies of the raw data, protocol and final report, along with all study correspondence can be destroyed after this time (see Section 9).

12. 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 7. Mortality, individual body weights, and cage-side and necropsy observations are presented in Tables 8 through 10.

The gravimetric and nominal chamber concentrations were 5.10 mg/L and 37.76 mg/L, respectively. The average mass median aerodynamic diameter was estimated to be 2.93 µm based on graphic analysis of the particle size distribution as measured with a 1 ACFM Andersen Ambient Particle Sizing Sampler with an average geometric standard deviation of 2.42.

One male and three females died within seven days of exposure to the test atmosphere. Prior to death, the animals were hypoactive and exhibited abnormal respiration and/or ano-genital staining. Following exposure, all surviving animals were hypoactive and exhibited abnormal respiration. In addition, several survivors exhibited ano-genital staining, ocular discharge, facial staining, and/or nasal discharge. Although all animals lost weight through Day 3, the animals gained body weight during Days 7 to 14. However, two of the survivors did not surpass their initial body weights by the end of the 14-day observation period. Gross necropsy of the decedents revealed discoloration of the lungs (red), distention of the stomach, discoloration of the liver and/or distention of the intestines. No gross abnormalities were noted for any of the surviving animals when necropsied at the conclusion of the 14-day observation period.

13. CONCLUSION

The red discoloration of the lung correlated with clinical findings of irregular respiration, rales (moist), nasal discharge, ocular discharge and/or gasping observed in all of the decedents. These findings are interpreted to be related with local irritative effects to the respiratory tract associated with test substance administration and to have contributed to the mortality observed in this study.

Under the conditions of this study, the single exposure acute inhalation LC_{50} of Ammonium Niobium Oxalate is greater than 5.10 mg/L in both sexes.

SIGNATURE

Ammonium Niobium Oxalate

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.

Daniel Merrill, BS, MBA
Study Director
Product Safety Labs

9/26/14
Date

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

1. Dust Generator:	Fluid Energy Jet Mill Serial #: J2724E
2. Air Supply:	Air Compressor (Powerex Model: SES050822)
3. Dust Delivery Apparatus:	Accurate Series 100 Dry Material Feeder
4. Chamber:	28 liter (Nose-Only Inhalation Chamber, ADG Developments LTD)
5. Compressed Generator Air Measurements:	Mass Flow Controller (Omega, Model #FMA-5541)
6. Compressed Mixing Air Measurements:	Mass Flow Controller (Omega, Model #FMA-5527)
7. Gravimetric Airflow Measurements:	Mass Flow Controller (Aalborg, Model #GFC-17)
8. Vacuum Pump:	GAST (Model #1531-107B-G557X)
9. Helix size:	1/2 inch

TABLE 2: PRE-TEST EXPOSURE TRIALS

Trial No. [1]	Generator/ Mixing Air Pressure (psi)	Compressed Generator Air (Lpm)	Compressed Mixing Air (Lpm)	Total Air Volume (Lpm)	Motor Setting	Chamber Conc. (mg/L)	Particle Size Sampled
1	50/30	50.0	10.0	60.0	4.0	2.88	No
2	50/30	50.0	10.0	60.0	7.0	13.08	No
3	50/30	50.0	10.0	60.0	5.0	4.00	No
4	50/30	50.0	10.0	60.0	5.2	4.50	No
5	60/30	60.0	10.0	70.0	5.4	5.28	Yes

[1]Trials used test substance ground in a coffee mill.

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

Trial No.	Chamber Concentration (mg/L)	Mass Median Aerodynamic Diameter[2] (µm)
5	5.28	2.98

[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 a 1 ACFM Andersen Ambient Sizing Sampler.

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	22.2	4	1	5.55
2	1	17.4	4	1	4.35
3	2	20.6	4	1	5.15
4	2.5	21.1	4	1	5.28
5	3.5	20.6	4	1	5.15
6	3.75	20.5	4	1	5.13
Average ± Standard Deviation					5.10 ± 0.40

TABLE 5: NOMINAL CHAMBER CONCENTRATION

Total Test Substance Used (g)	Average Total Airflow (Lpm)	Total Time of Exposure (min)	Nominal Concentration[1] (mg/L)
639.6	70.0	242	37.76

[1] Nominal Concentration = $\frac{\text{Total Test Substance Used (mg)}}{\text{Average Airflow x Total Time}}$

TABLE 6: PARTICLE SIZE DISTRIBUTION

Stage	Effective Cutoff Diameter (µm)	% of Total Particles Captured (by weight)	Cumulative (%)[1]
Sample 1			
0	9.0	6.1	93.9
1	5.8	10.8	83.1
2	4.7	8.1	75.0
3	3.3	20.4	54.6
4	2.1	24.4	30.2
5	1.1	21.1	9.1
6	0.7	5.5	3.6
7	0.4	2.0	1.7
F	0.0	1.7	0.0
Sample 2			
0	9.0	9.4	90.6
1	5.8	12.4	78.2
2	4.7	11.4	66.7
3	3.3	20.6	46.2
4	2.1	15.4	30.8
5	1.1	18.6	12.2
6	0.7	7.2	5.0
7	0.4	2.7	2.2
F	0.0	2.2	0.0

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

TABLE 7: SUMMARY OF PARTICLE SIZE DISTRIBUTION

Sample No.	Time of Sample (hours)	Collection Time (minutes)	Mass Median Aerodynamic Diameter[1] (µm)	Geometric Standard Deviation
1	1.5	1	2.80	2.32
2	3	1	3.06	2.51
		Average	2.93	2.42

[1]This figure is an estimation based on graphic analysis of the particle size distribution as measured with a 1 ACFM Andersen Ambient Sizing Sampler.

TABLE 8: INDIVIDUAL BODY WEIGHTS AND MORTALITY (5.10 mg/L)

Animal No.	Sex	Body Weight (g)					Mortality	
		Initial	Day 1	Day 3	Day 7	Day 14	Day	Weight (g)
3301	M	253	215	215	237	279	E	-
3302	M	267	229	201	216	236	E	-
3303	M	242	211	201	223	238	E	-
3304	M	248	211	192	-	-	7	178
3305	M	265	230	240	269	298	E	-
3306	F	193	169	155	168	197	E	-
3307	F	199	172	158	-	-	5	139
3308	F	179	157	-	-	-	2	149
3309	F	196	171	171	-	-	6	169
3310	F	170	163	167	173	183	E	-

E - Euthanized via CO_2 inhalation after weighing on Day 14

TABLE 9: INDIVIDUAL CAGE-SIDE OBSERVATIONS (5.10 mg/L)

Animal Number	Findings	Day of Occurrence
MALES		
3301	Rales (moist)	CR[1]-1
	Hypoactivity	CR-2
	Irregular respiration	CR-3, 5-11
	Gasping	0(2 hrs)-1
	Ano-genital staining	2
	Ocular discharge (black, left eye)	3-8
	Active and healthy	12-14
3302	Rales (moist)	CR-3
	Hypoactivity	CR-4
	Irregular respiration	CR-13
	Gasping	0(2 hrs)-1, 8-11
	Nasal discharge (red)	1-2
	Facial staining (black)	3-5
	Active and healthy	14
3303	Rales (moist)	CR-1
	Irregular respiration	CR-4
	Hypoactivity	0(1 hr)-1
	Active and healthy	5-14
3304	Rales (moist), irregular respiration	CR-6
	Hypoactivity	0(1 hr)-6
	Ano-genital staining	1-3
	Dead	7
3305	Hypoactivity	CR-0(2 hrs)
	Irregular respiration	CR-2
	Active and healthy	3-14

[1] CR - removal from the exposure tube

TABLE 9 (cont.): INDIVIDUAL CAGE-SIDE OBSERVATIONS (5.10 mg/L)

Animal Number	Findings	Day of Occurrence
FEMALES		
3306	Irregular respiration	CR-6
	Hypoactivity	0(1 hr)-3
	Rales (moist)	1-4
	Ano-genital staining	2-8
	Active and healthy	9-14
3307	Irregular respiration	CR-4
	Hypoactivity	0(1 hr)-2
	Rales (moist)	1-4
	Ano-genital staining	2
	Dead	5
3308	Irregular respiration, hypoactivity	CR-1
	Rales (moist)	1
	Dead	2
3309	Hypoactivity	CR-1, 6(am)
	Irregular respiration	CR-3, 5-6(am)
	Rales (moist)	1
	Ano-genital staining	1-2
	Active and healthy	4
	Dead	6(pm)
3310	Hypoactivity	CR-0(2 hrs)
	Irregular respiration	CR-2
	Active and healthy	3-14

TABLE 10: INDIVIDUAL NECROPSY OBSERVATIONS (5.10 mg/L)

Animal Number	Tissue	Findings
MALES		
3301-3303, 3305	All tissues and organs	No gross abnormalities
3304	Lungs	Extremely red
	Intestines	Slightly distended
FEMALES		
3306, 3310	All tissues and organs	No gross abnormalities
3307	Lungs	Slightly red
	Stomach, intestines	Slightly distended
3308	Lungs	Extremely red
	Liver	Mottled
	Stomach, intestines	Slightly distended
3309	Lungs	Slightly red
	Liver	Mottled

APPENDIX A: SUBSTANCE IDENTITY INFORMATION

knoell

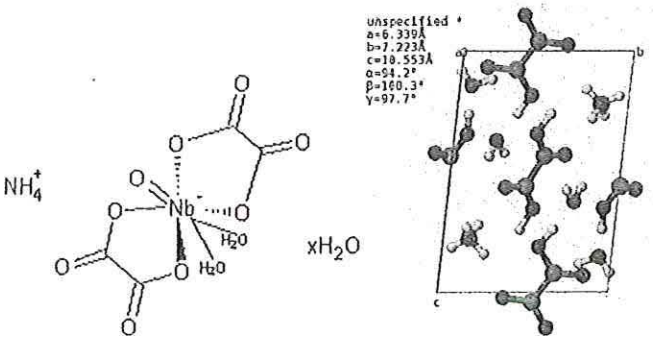
1. **Substance identity of ANO (CBMM)**

The substance ANO (Ammonium Niobium Oxalate, Sponsor CBMM) was examined. The following data according substance identity have to be indicated on "test item" in the study reports.

Test item:	ANO (common name)
Batch / Lot number:	AD/4663
Chemical name:	**Reaction mass of ammonium diaqua[bis(oxalate)]oxoniobate(1-) hydrate and ammonium hydrogen oxalate oxalic acid (1:1:1) dehydrate**
Type of substance:	Multi-constituent substance
Purity:	≥ 96%
Molecular weight range:	339.012 - 446.261

The reported molecular weight (MW) is indicated for the reaction mass, of which the constituent 1 contains crystal water x ranged from 0 to 8 ($NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O$). Also the MW of 339.012 refers to the constituent 1 (x=0) and 466.261 to the constituent 2.

Structural formula:



Main constituents:
Ca. 70% (68-74% (w/w)) constituent 1: $(NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O)$; x=0-8

knoell

Ca. 27% (24-28% (w/w)) constituent 2: $(NH_4(C_2HO_4) \cdot (C_2H_2O_4) \cdot 2(H_2O))$

Impurities:
Ca. 2.5% (1-3% (w/w)) free water
Ca.0.5% (0.1-1% (w/w)) organic and inorganic impurities (Na, K, Cl and SO4, as well as possible small quantity of reaction residue of oxalate and ammonium)

Constituent 1 $(NH_4[NbO(C_2O_4)_2 \cdot 2H_2O] \cdot xH_2O)$; x=0-8
IUPAC name: Ammonium oxobis(ethanedioato) bisniobate(V) hydrates
Molecular formula: C4H8NNbO11.xH2O, x= 0 – 8
Molecular weight range: 339.012 - 483.134 (MW range is calculated for crystal water range x= 0-8)

Constituent 2 $(NH_4(C_2HO_4) \cdot (C_2H_2O_4) \cdot 2H_2O)$
IUPAC name: Ammonium hydrogen ethanedioate ethanedioic acid dehydrate
Molecular formula: C8H9N2O16.4H2O
Molecular weight: 466.261

Appearance: white powder