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STUDY OF THE EFFECTS OF CHRONIC EXPOSURE
TO AEROSOLIZED POLYMER PARTICLES
ON THE RESPIRATORY SYSTEM

THE J. H. WILSON, JR. RESEARCH CENTER
FOR THE STUDY OF AIR POLLUTION
AND
DR. W. K. KORT, JR., PH.D., M.D., M.A. 1962

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DIVISION OF TOXICOLOGY

Toxicology Series: 191

ATMOSPHERIC AND ENVIRONMENTAL RESEARCH
INSTITUTE OF ENVIRONMENTAL SCIENCES, CALIFORNIA 94720

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John J. Beatrice 8 Jul 88

John J. Beatrice
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ABSTRACT

The potential of nitroguanidine (LAIR Code Number TP036A) to induce Sister Chromatid Exchanges (SCEs) was assessed using Chinese Hamster Ovary (CHO) cells both with and without exogenous metabolic activation provided by rat liver S-9. Cells were exposed to test compound concentrations ranging from 4 mg/ml to 0.01 mg/ml in cultures without exogenous metabolic activation and 3.9 mg/ml to 0.01 mg/ml in cultures with exogenous metabolic activation. Nitroguanidine did not induce a statistically significant increase in SCEs in either the presence or absence of exogenous metabolic activation. These results indicate that nitroguanidine was not an inducer of SCEs under the conditions of this study.

Key Words: DNA Damage, Genetic Toxicology, Sister Chromatid Exchange, Nitroguanidine



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PREFACE

TYPE REPORT: Sister Chromatid Exchange Assay GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Frederick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: #3E162720A835/180/TLB0

GLP STUDY NUMBER: 85036

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: MAJ John W. Harbell, PhD, MSC

REPORT AND DATA MANAGEMENT: A copy of the final report, retired SOFs, study protocol, retired stability and purity data on the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitroguanidine CAS # 556-88-7

INCLUSIVE STUDY DATES: 15 Jul 85 - 14 Jan 86

OBJECTIVE: The objective of this study was to determine the potential of nitroguanidine (TP036A) to induce sister chromatid exchanges by using CHO cells in the presence and absence of exogenous metabolic activation.

ACKNOWLEDGMENTS

Joanne Wong provided research assistance during this study.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS

We, the undersigned, declare that GLP study number 85036 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte, Jr. 15 Jun 88
DON W. KORTE, JR., PhD / Date
MAJ, MS
Study Director

John W. Harbell 8 June 88
JOHN W. HARBELL, PhD / Date
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Analytical Chemist



DEPARTMENT OF THE ARMY

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PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA (70-1n)

17 June 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85036

1. This is to certify that in relation to GLP Study 85036, the following inspections were made:

29 March 1985	- Protocol Review
27 August 1985	- Isolation and Fixation of Metaphase Cells

2. The institute report entitled "Sister Chromatid Exchange Assay of Nitroguanidine in Chinese Hamster Ovary Cells," Toxicology Series 191, was audited on 25 May 1988.

Carolyn M. Lewis
CAROLYN M. LEWIS
Chief, Quality Assurance

TABLE OF CONTENTS

Abstract	i
Preface	iii
Acknowledgments	iv
Signatures of Principal Scientists	v
Report of the Quality Assurance Unit	vi
Table of Contents	vii
BODY OF THE REPORT	
INTRODUCTION	1
Objective of the Study	2
MATERIALS AND METHODS	2
Test Compound	2
Chemical Preparation	3
Positive Controls	3
Cells	3
Medium	3
Metabolic Activation System	4
Assay Format	4
Data Evaluation	7
Changes/Deviations	7
Storage of Raw Data and Final Report	8
RESULTS	8
DISCUSSION	8
CONCLUSION	10
REFERENCES	11
APPENDIX	12
OFFICIAL DISTRIBUTION LIST	15

Sister Chromatid Exchange Assay of Nitroguanidine in Chinese Hamster Ovary Cells--Harbell et al.

INTRODUCTION

Nitroguanidine, ¹³a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine database and identified significant gaps in the toxicity data. ~~The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. This study evaluated the genotoxic potential of nitroguanidine by using the Sister Chromatid Exchange (SCE) Assay.~~

Exchanges between sister chromatids are detected by growing cells in the presence of bromodeoxyuridine (BrdU). Because DNA replication is semiconservative, cells grown with BrdU would contain chromosomes with substituted chromatids after one round of replication. The presence of BrdU allows for differential staining of chromatids with the fluorescence-plus-Giemsa (FPG) technique. If DNA damage occurred, then the damage corrected by post-replicative DNA repair processes would involve recombinational events (exchanges) between sister chromatids. The recombinational events are detected as a varied pattern of dark and light staining in the chromatid segments. The frequencies of these SCEs are analyzed since a direct correlation between the number of SCEs and the amount of DNA damage is assumed.

Many compounds can be converted into mutagenic agents by enzymes associated with normal metabolism. To detect these promutagens, the SCE assay is performed both with and without metabolic activation.

Barbell et al.

Objective of the Study

The objective of this study was to determine the potential of nitroguanidine (TP036A) to induce sister chromatid exchanges by using CHO cells both with and without exogenous metabolic activation.

MATERIALS AND METHODS

Nitroguanidine was evaluated for its potential to induce SCEs according to LAIR SOP, OP-STX-72 (2). All reagent concentrations listed in the following text are final concentrations unless specifically indicated otherwise.

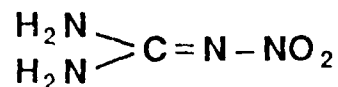
Test Compound

Chemical Name: Nitroguanidine

LAIR Code Number: TP036A

Chemical Abstracts Service Registry No.: 556-88-7

Structural Formula:



Empirical formula: CH₄N₄O₂

Storage: Nitroguanidine was obtained from Hercules Aerospace Division, Sunflower Army Ammunition Plant, DeSoto, Kansas (lot no. 20W94K101A001) and was assigned the LAIR Code number TP036A. The test compound was stored at room temperature in a bunker on the Presidio of San Francisco. Small amounts of test compound were stored at room temperature in the laboratory just before use.

Chemical Properties/Analysis: Data characterizing the chemical composition and purity of the test material were prepared by the Division of Toxicology, LAIR, Presidio of San Francisco, CA (Appendix).

Chemical Preparation

Nitroguanidine was dissolved directly in the cell culture medium (GEM 1717), without fetal bovine serum (FBS), at a concentration of 4.4 mg/ml and was filter-sterilized. This concentration is close to saturation for nitroguanidine in an aqueous medium (1). All test article dosing solutions were prepared from this stock solution. For the nonactivated cultures, FBS was added to the culture medium to achieve a final concentration of 10%. Dilutions were prepared using GEM 1717 with 10% FBS. For cultures with metabolic activation, two batches of cofactor mixture (see below) were prepared. One contained nitroguanidine while the other did not, and nitroguanidine dilutions were prepared by mixing the two solutions.

Positive Controls

The positive control for cultures without metabolic activation was ethyl methanesulfonate (EMS) diluted in sterile distilled water and added to the culture to achieve a final concentration of 0.320 mg/ml. The positive control for activated cultures was cyclophosphamide which was also dissolved in sterile distilled water and added to the culture to achieve a final concentration of 1.33 μ g/ml.

Cells

Chinese Hamster Ovary (CHO) Cells were obtained from Dr. Sheldon Wolff, University of California, San Francisco. The CHO cells were grown in an atmosphere of 5% CO₂ at 37 $\pm$ 1°C in GEM 1717 with 10% FBS. The cell cycle time was 10-12 hours. Twenty-four hours before treatment, 6 x 10⁵ cells were seeded into each 75 cm³ flask, which contained 15 ml of fresh medium, to ensure that the cells were in the logarithmic phase of growth when the test compound was added. Cells were tested and shown to be negative for mycoplasma (3).

Medium

HEPES buffered (10 mM) GEM 1717, which was used throughout, was prepared from RPMI 1640 (Gibco® Laboratories, Grand Island, NY) according to OP-STX-72 (2). Except when the medium was used to prepare the activation cofactor mixture (see below), it contained 10% (v/v) FBS when in contact with the cells.

Metabolic Activation System

The metabolic activation system was composed of Aroclor-induced rat liver 9000 x g supernatant fraction (S-9) (Lifton Bi-Science, Kensington, MD) and an NADPH-regenerating system provided by the cofactor mixture. The cofactor mixture consisted of NADP (2 mg/ml) and a reduced carbon source (sodium succinate, 4.5 mg/ml). The cofactors were dissolved in GEM 1717, without serum, and filter-sterilized. This solution was then supplemented to make the complete metabolic activation system which contained 2% FBS and 10% S-9. The metabolic activation system was prepared immediately before use.

Assay Format

Exposure:

The procedure used was a modification of the procedures developed by Perry and Evans (4) and Stetka and Wolff (5). The test compound as well as the positive and negative controls were evaluated both with and without metabolic activation using standard exposure periods (2). In tests performed without metabolic activation, the test compound was added to fresh GEM 1717 with 10% FBS (15 ml per flask) containing 5 μ M BrdU. After addition of the fresh medium, test compound, and BrdU, the cells were grown in the dark at $37\pm1^\circ\text{C}$ for 21.5 hours. Flasks were wrapped in foil to assure complete darkness. Then colchicine was added to each flask (0.4 μ g/ml). The cultures were incubated for an additional 2.5 hours before the cells were harvested.

In tests performed with metabolic activation, the complete medium was removed, and 15 ml of activation mixture, with or without the test article or positive control, was added. The cell cultures were exposed to the test compound in the activation mixture for 2 hours. This shorter exposure time in the presence of the activation mixture was used to preclude the occurrence of cytotoxic effects induced by the activation mixture. After the 2-hour exposure period, the medium containing both the test compound and the metabolic activation mixture was aspirated, the cells were washed three times with GEM 1717, and fresh, complete GEM 1717 containing 10% FBS and 5 μ M BrdU was added. The cultures were incubated in the dark for 21.5 hours. Colchicine was then added at a final concentration of 0.4 μ g/ml as described above and the

cultures were incubated for an additional 2.5 hours before the cells were harvested.

Harvest:

After 2.5 hr in colchicine, all of the cells in each culture were harvested. At harvest, the medium, which contained dividing cells, was removed from the culture flasks and saved. Each flask was rinsed once with 10 ml of Ca^{++} Mg^{++} Free Hank's Balanced Salt Solution (HBSS), and this wash was combined with the culture medium in a 50-ml tube. Ten ml of Ca^{++} Mg^{++} Free HBSS (with 0.02% EDTA) were added to the rinsed flasks, which were then shaken for approximately 30 min, until almost all the remaining cells were detached. While the flasks were shaking, the contents of each 50-ml tube were spun down (1000 rpm for 5 min), and all but 3 ml of the supernatant above each cell pellet was discarded. Each cell pellet was resuspended in its 3 ml of supernatant, and these cell suspensions were transferred to prelabelled 15-ml centrifuge tubes. Each tube was labelled with the protocol number, date of the experiment, and the culture (dose) number. The cell suspension from each flask was added to the appropriate tube (thus combining all the cells from a given flask), and the cells were centrifuged (1000 rpm for 5 min). Most of the supernatant was aspirated, and each cell pellet was gently resuspended in the remaining supernatant (approximately 0.2 to 0.3 ml/tube). Ten ml of hypotonic KCl (0.075 M) were added slowly (over about 2 min) to each tube, and the tubes were held at room temperature for approximately 2 min. The tubes were again centrifuged (1000 rpm for 5 min), the supernatant aspirated, and the cell pellet gently resuspended in the approximately 0.3 ml of remaining supernatant. Freshly prepared Carnoy's fixative (methanol:acetic acid 3:1) was then added slowly to each tube. The first 2 ml of fixative were added while gently mixing the resuspended cells. After a total of 8 ml had been added, the cells were allowed to fix for at least 10 min. The cells were again centrifuged into a pellet and the fixation procedure was repeated. The cells were allowed to fix overnight. In the morning, the cells were centrifuged and the fixation procedure was repeated. Before preparing the slides, the cells were centrifuged, all but about 0.5 ml (depending on the cell pellet size) of supernatant was removed, and the pellet was resuspended. Prelabelled clean slides were wetted in cold distilled water. Up to 4 drops of cell suspension were dropped onto each slide.

Staining:

The slides were stained by a modified FPG technique (5). Several drops of Hoechst 33258 (50 $\mu\text{g}/\text{ml}$ in water) were placed on each slide, and a coverslip was applied. After standing in the dark for 15 min, the coverslips were removed and the slides were washed several times in tap water and allowed to dry. Several drops of McIlvaine's buffer (pH 8) were placed on each slide, and a new coverslip was applied. These slides were placed on the $50\pm 2^\circ\text{C}$ warming table under a UV lamp. The slides were exposed to the UV light to induce photo-cleavage of the FNA strands (5). The coverslips were removed, and the slides were rinsed several times in tap water and dried. They were stained in dilute Giemsa stain (5% Guin's Giemsa pH 7.0), rinsed, and air dried; then a coverslip was applied.

Scoring:

For each dose group, triplicate slides were prepared and coded. The number of first-, second-, and third-division metaphases were scored (where possible, 100 metaphases were scored). Normally, 40 second-division metaphases, containing 20 chromosomes, were scored for SCEs in each preparation. In some cases, there were less than 40 second-division metaphases to score or the number of SCEs per cell was sufficiently high so that fewer cells needed to be counted to achieve statistical significance. In the first case, all available metaphases (with 20 chromosomes) were scored on all slides.

Raw Data Collection:

All data were collected from coded slides. The data collected included slide number (coded), quality of the slide (as needed), and the number of first-, second-, and third-division metaphases. For each metaphase scored, the following data were collected: the number of chromosomes, the number of SCEs, the vernier settings, and the photograph number. Each metaphase scored for SCEs was photographed to allow positive identification of that metaphase during rescoring. Approximately 50% of the scored metaphases were rescored by an independent scorer. After scoring of the experiment was completed, the slides were decoded and the slide number, the percentage of first- and second-division metaphases, and SCEs per cell were matched to the treatment groups for further analysis.

Statistical Analysis:

Statistical evaluation of the SCE data was a two-step process involving the use of a one-way analysis of variance to compare variance within treatments (due to scoring differences and sample difference) with variance among treatments (the effect of test compound) followed by a Student-Newman-Keuls test to indicate which groups (treatments) were different. Thus, the ANOVA was used to indicate a difference among groups and the Student-Newman-Keuls test was used to indicate which specific dose or group of doses was different from each other or controls. The positive and negative controls were compared by using the t-test. The $p \leq 0.05$ confidence limit was used for the t-test, ANOVA, and Student-Newman-Keuls test.

Data Evaluation

Criteria for a Valid Test:

A valid SCE assay is one in which (1) testing was performed to the maximum concentration expected to yield sufficient second-division metaphases for analysis, the limits of solubility, or the limits of compound availability (a maximum of 5000 $\mu\text{g/ml}$ or 0.5% for liquids), and (2) the positive control induced a statistically significant increase in the SCE frequency.

Interpretation of Results:

Positive: A test compound will be considered to have elicited a positive response in the SCE assay if it induces a statistically significant ($p < 0.05$) increase in the SCE frequencies compared to the negative control rate (by the Student-Newman-Keuls test) and the SCE frequency exhibited a correlated dose response.

Negative: A test compound will be considered to have elicited a negative response in the SCE assay if the criteria (statistical significance or dose response) for a positive response are not met.

Changes/Deviations

The actual conduct of the study differed slightly from the SOP during the harvesting phase. Instead of shaking each rinsed flask for 10 min with HBSS, the flasks were shaken for

30 min. This provided sufficient time for the cells still adhering to the flask to detach.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Nitroguanidine was tested over a concentration range of 4.0 to 0.01 mg/ml without exogenous metabolic activation and 3.9 to 0.01 mg/ml with activation. Specific doses are given in Table I. Nitroguanidine did induce some toxicity. At the highest dose without metabolic activation (4.0 mg/ml), 91% of the metaphases isolated were found to be from first-division cells, and the total yield of metaphases was too small for SCE scoring. The 2 mg/ml dose induced some toxicity during the 24-hour exposure but failed to induce a significant rise in SCEs. None of the lower doses of nitroguanidine induced toxicity or increases in SCEs. The assay with metabolic activation used a 2-hour exposure period. In this case, neither the highest dose (3.9 mg/ml) nor the lower ones induced any appreciable toxicity or increase in SCEs.

Both positive controls induced statistically significant increases in SCEs.

DISCUSSION

Nitroguanidine was tested for its ability to induce SCEs in CHO cells both with and without exogenous metabolic activation. Even at the highest doses tested, nitroguanidine did not induce a statistically significant increase in SCEs nor was there an apparent dose-dependent increase in SCEs. The highest dose tested was dictated by the limits of solubility. Both positive controls induced significant increases in the SCEs. Thus, all the conditions for a valid, negative assay were met.

Nitroguanidine has been reported to cause significant chromosomal damage in Chinese hamster fibroblasts (6). Ishidate and Odashima used a dose of 4 mg/ml for 24 hours and found 26% of the metaphases to have chromosomal aberrations, principally gaps, breaks, and translocations. Our data from

Table I: NITROGUANIDINE (NG) SISTER CHROMATID EXCHANGE

DOSES		%FD ^a	n ^b	SCE	±1SD	COMMENT
WITHOUT S-9 (24-hour exposure)						
Neg Control		2	40	6.58	±2.86	
EMS	0.32 mg/ml	12	6	61.00	±9.74	p<0.0001
NG	4.0 mg/ml	91	40	metaphases not scorable		
NG	2.0 mg/ml	14	40	7.28	±2.53	NS
NG	1.0 mg/ml	3	40	7.30	±2.66	NS
NG	0.5 mg/ml	2	40	7.20	±3.03	NS
NG	0.1 mg/ml	1	40	6.78	±2.83	NS
NG	0.05 mg/ml	7	40	6.25	±2.26	NS
NG	0.01 mg/ml	4	40	7.25	±2.65	NS
WITH S-9 (2-hour exposure)						
Neg Control		1	39	9.31	±2.57	
Cyclophosphamide						
	1.33 µg/ml	9	20	34.5	±11.75	p<0.0001
NG	3.9 mg/ml	1	39	9.03	±3.27	NS
NG	2.0 mg/ml	1	19	6.53	±2.57	NS
NG	1.0 mg/ml	2	28	8.00	±3.10	NS
NG	0.5 mg/ml	1	40	7.85	±3.31	NS
NG	0.1 mg/ml	4	20	8.60	±3.22	NS
NG	0.05 mg/ml	0	40	8.65	±3.32	NS
NG	0.01 mg/ml	4	40	7.00	±2.66	NS

^a %FD = percentage of metaphases in first division

^b n = number of metaphases scored for SCEs

Negative controls and nitroguanidine groups were compared using ANOVA.

Negative controls and positive controls were compared using the t-test.

Abbreviations: EMS = ethyl methanesulfonate, NG = nitroguanidine, SCE = sister chromatid exchange, SD = standard deviation, NS = not significant (P≤0.05).

the SCE assay showed NG to be very toxic at that dose over a 24-hour exposure. Most of the metaphases detected were from first-division cells, and even those that showed some differential staining had not gone through two complete replication cycles in the BrdU-containing medium. Rather, these cells appeared to have been in S phase when the nitroguanidine and BrdU were added and went on to complete this cycle. After the second S phase, these cells contained a mixture of thymidine, thymidine and BrdU, and BrdU-containing strands so that they were not scorable for SCEs. Examination of the metaphases from this dose group did not reveal the chromosomal aberrations reported previously. Thus our data are at odds with those of Ishidate and Odashima (6) though the test systems were by no means identical (e.g. different cell lines, presence of BrdU, and different chromosome preparation). In addition, no specific chemical purity data were provided (6).

Nitroguanidine was also found to be negative in the Ames Salmonella/Microsome assay and Mouse Lymphoma TK $\pm$ Forward Mutation assay (7). In these studies, nitroguanidine was tested to the limits of solubility for each assay system both with and without exogenous metabolic activation.

CONCLUSION

Nitroguanidine was not a significant inducer of SCEs under the conditions of this assay either with or without exogenous metabolic activation.

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Appendix: CHEMICAL DATA

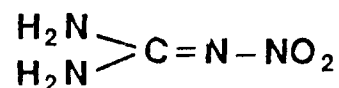
Chemical name: Nitroguanidine (NG)

Other listed names: Guanidine, Nitro; alpha-Nitroguanidine;
beta-Nitroguanidine

Chemical Abstracts Service Registry No.: 556-88-7

LAIR Code: TP036A

Structural formula:



Molecular formula: CH₄N₄O₂

Molecular weight: 104.1

pH range of dosing suspensions: 6.7 - 7.4⁽¹⁾

Physical state: White Powder

Melting point: 232°⁽²⁾

Source: Hercules Aerospace Division
Sunflower Ammunition Plant
DeSoto, Kansas

Lot No. SOW84K010A001

Purity: 99.2% (data sheet attached)

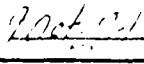
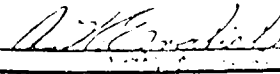
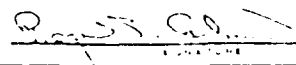
1. Wheeler CR. Nitrocellulose-Nitroguanidine Projects
Laboratory Notebook #85-12-022, p 26. Presidio of San
Francisco, CA: Letterman Army Institute of Research
2. Fedoroff BT, Sheffield OE. Encyclopedia of explosives
and related items. Vol V. Dover, New Jersey: Picatinny
Arsenal 1975: G154.

Appendix (cont.): CHEMICAL DATA

Analytical data: The major peaks in the infrared spectrum of the compound were observed at 3450, 3396, 3342, 3278, 3201, 1666, 1634, 1525, 1404, 1314, 1151, 1045, 782 cm^{-1} (3). The spectrum obtained for the test compound in our laboratory was identical to the Sadtler standard spectrum for nitroguanidine (4). HPLC showed only one peak (retention time 4.9 min) (5). The conditions employed were as follows: column, Brownlee RP-18 (4.6 x 250 mm); solvent 10% methanol/90% water, flow rate 0.7 ml/min; oven temperature, 50°C; monitoring wavelength, 265 nm.

3. Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-12-022, p. 22-23. Presidio of San Francisco, CA: Letterman Army Institute of Research.
4. Sadtler Research Laboratory, Inc. Sadtler standard spectra. Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infra-red spectrogram #21421.
5. Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-12-022, pp. 24-25. Presidio of San Francisco, CA: Letterman Army Institute of Research.

Appendix (cont.): CHEMICAL DATA

DESCRIPTION SHEET FOR EXPLOSIVES, CHEMICALS, ETC				FOR CONTROL SYMBOL	PAGE
CRSAP-P-702-1091				EXEMPT-Para 7-2e AR 335-15	1 OF 1
TO: Commander US Army Armament Munitions and Chemical Command Attn: DRSMC-QAD Rock Island, ILL. 61299		FROM: Sunflower Army Ammunition Plant DeSoto, Kansas 66018		DATE: October 8, 1984	
				MATERIAL: Nitroguanidine	
MANUFACTURER: Hercules Aerospace Division, Hercules Inc.		CONTRACT NO.: DAAA-04-77-C-4016		CLIN 0045	
SECTION A - DESCRIPTION OF LOTS					
FROM NUMBER: SKW84K010A001	THRU NUMBER: -	TOTAL NO. LOTS: 1	TOTAL NET AMOUNT ACCEPTED: 25 pounds		
PLACE MANUFACTURED: Sunflower Army Ammunition Plant		SPECIFICATION AND AMENDMENT: CHARGING: MIL-N-004948 Std. 17 July 1954			
SECTION B - DESCRIPTION OF MATERIAL					
Requirement		Analysis *			
Property	Min.	Max.			
Purity, %	99.0		99.20		
Ash Content, %		0.30	0.12		
pH Value	4.5	7.0	6.0		
Acidity (as H ₂ SO ₄), %		0.06	0.0		
Total Volatiles, %		0.25	0.16		
Sulfates (as N ₂ SO ₄), %		0.20	0.17		
Impurities, H ₂ O Insoluble, %		0.20	0.02		
Particle Size, microns	3.4	6.0	3.5		
Color			White		
Consistency			Crystalline, Free Flowing		
* Combined averages of sampling taken in accordance with MIL-N-004948, Para. 4.4.3.2.					
REMARKS:					
1) Packaging: Level C Fiber drums per specification DOT 21C60					
2) This lot was manufactured 5 October 1984 and is submitted as First Article in compliance with Paragraph 4.3 of MIL-N-004948.					
3) Guanidine Nitrate supplied by SKW-American Boesch was used in manufacture of this lot.					
SECTION C - CERTIFICATION					
SAMPLING CONDUCTED BY: Hercules Aerospace Division		THE ABOVE MATERIAL COMPLIES WITH ALL SPECIFICATION REQUIREMENTS AND IS CERTIFIED TRUE AND CORRECT.			
TESTING CONDUCTED BY: Hercules Aerospace Division		 			
THE ABOVE DESCRIBED LOTS ARE HEREBY ACCEPTED		FOR THE COMMANDER			
10 OCT 1984					

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