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INSTITUTE REPORT NO. 197

MUTAGENIC POTENTIAL OF GUANIDINE HYDROCHLORIDE (TPO28)

STEVEN K. SANO, BA, SP4  
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and  
DON W. KORTE JR, PhD, MAJ MSC

TOXICOLOGY GROUP  
DIVISION OF RESEARCH SUPPORT

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Toxicology Series 80

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**Mutagenic Potential of: Guanidine Hydrochloride (TP028) (Toxicology Series 80)-- Sano, Kellner and Korte**

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1. REPORT NUMBER <b>LAIR Institute Report No. 197</b>	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) <b>Mutagenic Potential of: Guanidine Hydrochloride (TP028)</b>	5. TYPE OF REPORT & PERIOD COVERED <b>Final 6 - 27 February 1984</b>	
	6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s) <b>Steven K. Sano, BA, SP4 Thomas P. Kellner, BA, SP5 Don W. Korte, Jr., PhD, MAJ MS</b>	8. CONTRACT OR GRANT NUMBER(s)	
9. PERFORMING ORGANIZATION NAME AND ADDRESS <b>Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800</b>	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS <b>3E162720A835, WU 180</b>	
11. CONTROLLING OFFICE NAME AND ADDRESS <b>US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701-5012</b>	12. REPORT DATE <b>2 March 1985</b>	
	13. NUMBER OF PAGES <b>25</b>	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) <b>UNCLASSIFIED</b>	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)  <b>Mutagenicity, Toxicology, Ames Assay, Guanidine Hydrochloride (TP028)</b>		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)  <b>The mutagenic potential of guanidine hydrochloride (TP028) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 ml/plate to 0.0016 mg/plate. Negative mutagenic responses were observed for the test compound.</b>		

ABSTRACT

↙ The mutagenic potential of guanidine hydrochloride (TP028) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate. Negative mutagenic responses were observed for the test compound.

Key Words: Mutagenicity, Toxicology, Ames Assay, Guanidine Hydrochloride (TP028),

A

**PREFACE**

**TYPE REPORT:** Ames 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 Medical Bioengineering Research and  
Development Laboratory  
Fort Detrick, MD 21701-5012

**WORK UNIT:** 3E162720A835 Nitrocellulose-Nitroguanidine  
Projects; WU 180; APC TL09

**GLP STUDY NUMBER:** 84007

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

**PRINCIPAL INVESTIGATOR:** SP4 Steven K. Sano, BA

**CO-PRINCIPAL INVESTIGATOR:** SP5 Thomas P. Kellner, BA

**REPORT AND DATA MANAGEMENT:** 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.

**TEST SUBSTANCE:** Guanidine Hydrochloride (TP028)

**INCLUSIVE STUDY DATES:** 6 - 27 February 1984

**OBJECTIVE:** The objective of this study was to determine the mutagenic  
potential of guanidine hydrochloride (LAIR code TP028).

## ACKNOWLEDGMENTS

The authors wish to thank SP5 Lawrence Mullen, BS, and John Dacey for their assistance in performing the research.

Signatures of Principal Scientists Involved  
in the Study

We, the undersigned, believe the study number 84007 described  
in this report to be scientifically sound and the results and  
interpretation to be valid. The study was conducted to comply, with the  
Good Laboratory Practice Regulations outlined by the Food and Drug  
Administration.

*Don W. Korte, Jr.* *14 May 54*  
DON W. KORTE, JR., Ph.D. / DATE  
MAJ, MSC  
Study Director

*Steven K. Sano* *14 May 54*  
STEVEN K. SANO, B.A. / DATE  
SP4, USA  
Principal Investigator

*Thomas P. Kellner* *14 May 54*  
THOMAS P. KELLNER, B.A. / DATE  
SP5, USA  
Co-Principal Investigator



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31 Jul 84

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 84007 the following inspections were made:

22 Feb 84

The report and raw data for this study were audited on 23 Apr 84.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the 31 Mar 84 report to Management and the Study Director.

  
NELSON R. POWERS, Ph.D.  
DAC  
Chief, Quality Assurance Unit

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**Mutagenic Potential of: Guanidine Hydrochloride (TP028)**

-- Sano et al

The Ames Salmonella/Mammalian Microsome Mutagenicity Assay is a short-term screening assay that utilizes histidine auxotrophic mutant strains of Salmonella typhimurium to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the assay to increase sensitivity by simulating in vivo metabolic activation of the test compound. The Ames assay is an inexpensive yet highly predictive and reliable assay for detecting mutagenic activity and thus carcinogenic potential (1).

Objective of the Study

The objective of this study was to determine the mutagenic potential of guanidine hydrochloride (LAIR code TP028).

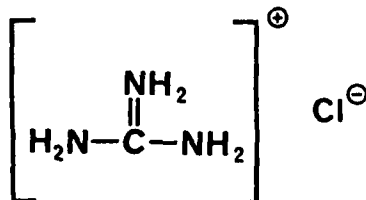
## METHODS

Test Compound

Chemical name: Guanidine Hydrochloride

Chemical Abstract Service Registry No.: 50-01-1

Structural formula:



Empirical formula:  $\text{CH}_5\text{N}_3\cdot\text{HCl}$

Storage: One kilogram of guanidine hydrochloride (Lot Number 103-F-5623) was obtained from the Sigma Chemical Co. (St. Louis, MO) in December 1983 and assigned the LAIR Code number TP028. The test compound was stored at room temperature (21°C).

Chemical Properties/Analysis: Data characterizing the chemical composition was obtained from Sigma Chemical Co. (St. Louis, MO) and the stability analysis of the test material was performed by the Toxicology Services Group, LAIR (Presidio of San Francisco, CA), (Appendix A).

#### Test Solvent

Positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 100F-0269) obtained from Sigma Chemical Co. (St. Louis, MO).

Test compound was dissolved in sterile, deionized water obtained from a Polymetrics Model 200-3 Water Purifier (Sunnyvale, CA).

#### Chemical Preparation

On the day before dosing, 300 mg of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 300 mg sample was dissolved in a 6 ml volume of autoclaved water from a polymetrics water filter system to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates. The dosing procedure was completed within 20 minutes of dissolving the test compound.

#### Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, were used. These strains were maintained in our laboratory at -80°C. Quality controls were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains, their genetic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (2).

#### Test Format

Guanidine hydrochloride was evaluated for mutagenic potential according to the methods of Ames et al (3). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (2).

### Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of guanidine hydrochloride ranging from  $1.6 \times 10^{-3}$  mg/plate to 5 mg/plate, and approximately  $10^8$  cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin were placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed decreased macrocolony formation (below the level of the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 mg per plate was used in the mutagenicity assay.

### Mutagenicity Assay

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5 both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and in all reagents came from a polymetric system. Plates were incubated, upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). No vehicle control was used as the test compound was diluted in water. The spontaneous reversion rate (with and without S-9) was monitored by averaging the count from two determinations run simultaneously with the test compound assay. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound assay plates so that any change in spontaneous reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames et al (3). Concurrent sterility and strain verification controls were run. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The Salmonella strains were verified by a standard battery of tests.

The following tests were run to determine if:

- Lipopolysaccharide layer (LP) alteration causes growth inhibition in the presence of crystal violet.
- An ampicillin-resistant R factor has allowed growth in strains TA 98 and TA 100 in the presence of ampicillin impregnated disks.
- Absence of excision repair mechanism has inhibited growth in the presence of ultraviolet light.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. These compounds, benzo [a] pyrene, 2-aminofluorene, 2-aminoanthracene and N<sup>1</sup> methyl-n-nitro-n-nitrosoguanidine, were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

#### Data Interpretation

According to Ames et al (3), a compound is considered mutagenic if the following criteria are met:

- The test compound causes revertant colony counts greater than or equal to twice the spontaneous revertant rate.
- The test compound produces a correlated dose response relationship.

#### RESULTS

On 15 February 1984, the toxicity level determination was performed on guanidine hydrochloride (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 2). No toxicity was observed after exposure of the tester strain (TA100) to the highest dose used (5 mg/plate).

Normal results were obtained for all sterility, strain verification, and negative controls during the Ames Assay performed on 21-23 February 1984 (Tables 3-4). No revertant counts were obtained that exceeded double the spontaneous reversion rate following exposure of the bacterial strains to the test compound (Table 5).

TABLE 1

TOXICITY LEVEL DETERMINATION

Substance assayed: GUANIDINE HYDROCHLORIDE Substance dissolved in: H<sub>2</sub>O  
 Study Number: 84007 Date: 17 February 1984 Performed by: MULLEN, KELLNER

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1			Plate #2			Plate #3			Average	Background Lawn (1)
	107	109	94	124	114	104	124	114	104		
5 mg/pl										103	NL
1 mg/pl										114	NL
0.2 mg/pl										114	NL
0.04 mg/pl										106	NL
0.008 mg/pl										114	NL
0.0016 mg/pl										105	NL

(1) NC = No Growth    ST = Slight Growth    NL = Normal Lawn

TABLE 2

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	NG (20 mm)	NG	+
Wild Type	G	NT	G	NT	NT	+

STERILITY CONTROL FOR TOXICITY LEVEL DETERMINATION

His-Bio Mix Initial: NG End: NG MGA Plate: NG  
 Top Agar Initial: NG End: NG  
 Diluent: NG Nutrient Broth: NG  
 Test Compound (a) NG (b)      (c)      (d)      (e)     

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9

(1) + = expected response - = unexpected response

Study Number: 24007 Date: 16 Feb 1984 By: KELLNER, SANO

TABLE 3

STRAIN VERIFICATION CONTROL FOR ASSAY

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	NG (20 mm)	NG	+
100	NG	G	NG	NG (25 mm)	NG	+
1535	NG	NT	NG	NG (26 mm)	NG	+
1537	NG	NG	NG	NG (26 mm)	NG	+
1538	NG	NT	NG	NG (25 mm)	NG	+
NT	NT	NT	G	NT	NT	+

STERILITY CONTROL FOR ASSAY

His-Bio Mix Initial: NG End: NG Diluent: NG  
 Top Agar Initial: NG End: w S-9 w/o S-9 MGA Plate: NG  
 S-9 Mix Initial: NG End: NG Nutrient Broth: NG  
 Test Compound (a) NG (b)        (c)        (d)        (e)        (f)         
 G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type  
 Study Number: 84007 By: SANO, KELLNER (1) + = expected response  
 Date: 23 February 1984 - = unexpected response

TABLE 4

POSITIVE AND NEGATIVE CONTROL TEST

Compd. *	Amount of Compd. Added	S-9 Added	(Revertants/Plate)			Strain No.
			98	100	1535	
			Mean			
AF	2 ug/plate	yes	(467,416,230) 371	(292,268,261) 267	1537	(348,308,326) 327
BP	2 ug/plate	yes	(104,114,100) 106	(598,650,640) 629	( 89, 67, 70) 75	(124,100,155) 126
AA	2 ug/plate	yes	(1397,1489,1331) 1406	(1643,1547,1621) 1604	(188,173,100) 154	(955,1187,1122) 1088
MNNG	2 ug/plate	no		(1607,1519,1520) 1549		
	20 ug/plate	no		(1556,1576,1578) 1570		

Spontaneous Reversion Rate/ Negative Control

before assay	yes	( 32, 30, 30)	(139,114,140)	( 18, 19, 19)	( 12, 8, 5)	( 12, 23, 19)
after assay		( 26, 26, 17)	(126,133,126)	( 25, 16, 18)	( 5, 4, 6)	( 17, 14, 15)
		27	130	19	7	17
before assay	no	( 20, 15, 23)	(126,112,116)	( 36, 28, 36)	( 6, 6, 8)	( 11, 15, 12)
after assay		( 25, 10, 14)	(142, 84,108)	( 25, 18, 16)	( 2, 4, 8)	( 14, 13, 15)
		18	115	27	6	13

Study Number: 84007

Date: 24 Feb 1984 By: KELLNER, SANO

\* Compounds: AF = 2-aminofluorene, BP = Benzo(a)pyrene, AA = 2-aminoanthracene,  
MNNG = N-methyl-n'-nitro-n-nitrosoguanidine

†\$\$\$ Indicates colony counts exceeded 1000

TABLE 5

GUANIDINE HYDROCHLORIDE ASSAY

Compd	Amount of Compd. Added	S-9 Added	(Revertants/Plate) Mean	Strain No.			
				98	100	1535	1537
TF028	5 mg/pl	yes	( 28, 40, 29) 32	(118,136,131) 128	( 19, 20, 25) 21	( 6, 6, 4) 5	( 18, 11, 17) 15
		no	( 26, 21, 18) 22	(122,139,120) 127	( 30, 28, 31) 30	( 5, 8, 6) 6	( 10, 7, 10) 9
	1 mg/pl	yes	( 36, 29, 24) 30	(133,125,173) 144	( 22, 21, 27) 23	( 8, 8, 5) 7	( 19, 15, 26) 20
		no	( 17, 23, 14) 18	(144,111,105) 120	( 34, 31, 26) 30	( 9, 4, 6) 6	( 14, 15, 14) 14
	0.2 mg/pl	yes	( 40, 27, 26) 31	(137,150,134) 140	( 20, 17, 14) 17	( 8, 5, 3) 5	( 10, 19, 23) 17
		no	( 21, 15, 15) 17	( 19, 25, 141) 62	( 6, 18, CON) 12	( 4, 7, 3) 5	( 10, 9, 9) 9
	0.04 mg/pl	yes	( 35, 33, 17) 28	(112,132,114) 119	( 19, 14, 16) 16	( 6, 6, 8) 7	( 13, 12, 16) 14
		no	( 18, 25, 15) 19	(156,112,140) 136	( 46, 52, 46) 48	( 6, 4, 9) 6	( 11, 11, 11) 11

Study No.: 84007 Date: 24 Feb 1984 Performed by: SANO, KELLNER

TABLE 5 (concluded)

GUANIDINE HYDROCHLORIDE ASSAY

Compd	Amount of Compd. Added	S-9 Added	(Revertants/Plate)		Strain No.		
			Mean	98	100	1535	1537
TPO28	0.008 mg/pl	yes	( 29, 28, 45)	(124,106,126)	( 22, 16, 13)	( 8, 3, 11)	( 21, 26, 20)
			34	119	17	7	22
		no	( 16, 19, 26)	(127,108,102)	( 34, 27, 36)	( 5, 4, 3)	( 11, 15, 13)
			20	112	32	4	13
	0.0016 mg/pl	yes	( 28, 24, 25)	(126,110,127)	( 17, 19, 14)	( 5, 10, 9)	( 23, 9, 32)
			26	121	17	8	21
		no	( 18, 17, 8)	(140,123,138)	( 41, 45, 31)	( 3, 8, 6)	( 22, 12, 12)
			14	134	39	6	15

Study No.: 84007

Date: 24 Feb. 1984 Performed by: SAND, KELLNER

## DISCUSSION

Certain test criteria must be satisfied before an Ames assay can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, LP layer alterations, and DNA excision repair deficiencies. Second, the Salmonella strains must be responsive to the mutagenic process by exposing the strains to known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolony and microcolony formation. If these tests are performed and expected data are obtained, then the results of Ames test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, guanidine hydrochloride was evaluated by the Ames assay. In no instance did the test compound elicit a doubling of the spontaneous reversion rate or a correlated dose response relationship. Thus, the results of this study indicate guanidine hydrochloride is not mutagenic when evaluated in the Ames assay.

## CONCLUSION

Guanidine hydrochloride is not mutagenic by the Ames assay at the dose levels tested.

## RECOMMENDATION

Guanidine hydrochloride should undergo further toxicity testing in accordance with the Toxic Substances Control Act.

REFERENCES

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2. Ames Salmonella/Mammalian Microsome Mutagenicity Assay. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.
3. Ames, BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian microsome mutagenicity test. Mutation Res 1975;31:347-364.
4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.

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APPENDIX

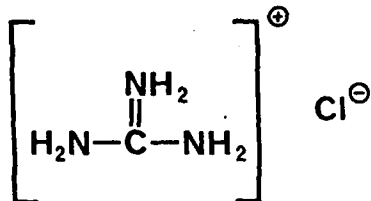
## CHEMICAL DATA

Chemical Name: Guanidine Hydrochloride

Alternate Chemical Name: Aminomethanimidine hydrochloride,  
Carbamimidine hydrochloride,  
Carbamide hydrochloride,  
Aminoformimidine hydrochloride,  
Iminourea hydrochloride

Chemical Abstracts Service Registry No.: 50-01-1

Chemical structure:



Molecular formula:  $\text{CH}_6\text{ClN}_3$

Molecular weight: 95.5

Physical state: White powder

Melting point: 182-184°C (184-185°C\*)

Analytical data/purity: Water content 0.1% by Karl Fischer analysis.\*  
The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.†  
Elemental analysis. Calculated for  $\text{CH}_6\text{ClN}_3$   
Cl, 37.1. Found: Cl, 36.6.† An IR spectrum was obtained upon receipt of the compound.  
IR(KBr): 3400, 2750, 1650, 1535, 1050 (broad)  $\text{cm}^{-1}$ .  
A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.‡

Source: Sigma Chemical Co.  
St. Louis, MO

Lot number: 103F-5623

\* Zygumt R., Analytical data sheet for guanidine hydrochloride, lot number 103F-5623. Sigma Chemical Co., St. Louis. 16 Feb 84.

† Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

‡ Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

Stability in vehicle: A preliminary study was conducted to determine the stability of guanidine hydrochloride in the vehicle, sterile water for injection. A solution of guanidine hydrochloride (18.825 ug/ml water) was assayed after preparation and 4 hours later by using the Voges-Proskauer Method (Micklus MJ, Stein IM. The colorimetric determination of mono- and disubstituted guanidines. Anal Biochem 1973;54:545-553). This method is specific for unsubstituted and monosubstituted guanidines and yields a colored derivative which is monitored spectrophotometrically. Three samples were analyzed for each time point and the results were as follows:

Absorbance Value <u>(1st Assay)</u>	Absorbance Value <u>(2nd Assay)</u>
2.190	2.053
2.165	2.190
2.160	2.191
$x = 2.172$	$x = 2.145$

The values for the two assays were within 1.5 percent of each other which is within the error for repeated sampling using this test. This indicates that the compound is stable in aqueous solution for at least 4 hours.

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**END**

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