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MICROBIOLOGICAL AND CHEMICAL TRANSFORMATIONS OF NITROGUANIDINE

By

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Science and Advanced Technology Laboratory

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Nitroguanidine was cometabolized under reducing conditions to nitrosoguanidine after acclimation. No further microbial reduction occurred (no aminoguanidine, hydrazine, or urea were detected in culture extracts). Nitrosoguanidine decomposed nonbiologically and formed cyanamide, cyanoguanidine, melamine and guanidine. All products were identified by TLC and GC/MS. A pathway for the degradation of nitroguanidine is proposed. No ammeline, ammelide or cyanuric acid were detected. Nitroguanidine and nitrosoguanidine were sensitive to UV light.

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PREFACE

Nitroguanidine, a military propellant, enters waste streams at handling facilities. Inadequate information is available in the literature to assess the potential problems nitroguanidine may pose in the environment, including any biohazards. Therefore, compliance with current pollution guidelines can not be addressed until further information is available. This report documents the fate of nitroguanidine upon exposure to microbiological activity and describes environmental concerns for contamination of wastewater by nitroguanidine. The work was conducted under project No. 103210502007. We thank Carmine Di Pietro for his GC/MS analysis.

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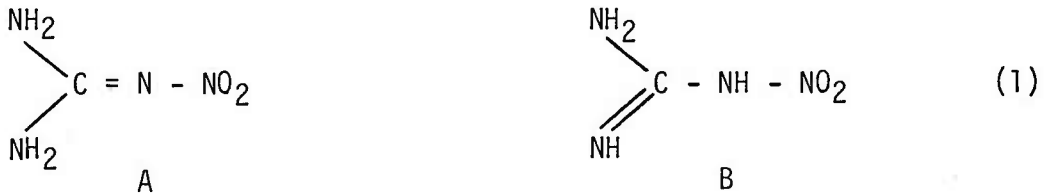
MICROBIOLOGICAL AND CHEMICAL TRANSFORMATIONS OF NITROGUANIDINE

INTRODUCTION

Nitroguanidine is used as a component of military propellants. It is water-soluble and substantial quantities may enter the environment via discharge streams from handling facilities.[1] [2] It is therefore essential to assess the extent to which nitroguanidine persists in the biosphere and the magnitude of the biohazard it presents. Insufficient information is available in the literature on the biological fate of nitroguanidine to assess these concerns.

Nitroguanidine is a nitroimino compound which exists in two tautomeric forms, Eq. 1.



(1)

Form A predominates in acidic, neutral, or slightly basic media.[3]

The purpose of this investigation was to evaluate the susceptibility of nitroguanidine to microbiological degradation. A further object was to gain insight on its persistence in the environment and the feasibility of biological treatment of nitroguanidine-containing waste streams.

[1] Bissett, F. H. and L. A. Levasseur. 1976. Analytical Methods for Nitroguanidine and Characterization of its Degradation Products. Technical Report TR-76/47. U.S. Army Natick Research and Development Command, Natick, MA.

[2] Small, M. J. and D. H. Rosenblatt. 1974. Munitions Production Products of Potential Concern as Waterborne Pollutants - Phase II. U.S. Army Bioengineering Research and Development Laboratory, Aberdeen Proving Ground, MD. Tech. Rpt. 7404.

[3] Kemula, W., M. K. Kalinowski, T. M. Krygowski, J. A. Lewandowski, and A. J. Walasek. 1970. Investigation of N-Nitroderivatives. Equilibria of Nitrourea and Nitroguanidine in Aqueous Solutions. Bull. Acad. Pol. Sci., Chem. Ser., 18: 445-461.

MATERIALS & METHODS

Media. Basal salts contained 1.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$ and 0.01 g NaCl per liter of distilled water. $NH_4H_2PO_4$, 2.0 g/L, and glucose, 1.0 g/L, were added as indicated. Nutrient broth concentrations ranged from 0.8 to 8.0 g/L.

Culture Conditions. Aerobic batch cultures were incubated in 250-ml Erlenmeyer flasks each containing 100 ml of media at 30°C on a New Brunswick G24 Environmental Incubator Shaker. Anaerobic batch cultures were incubated at 37°C in 250-ml Erlenmeyer flasks filled with media.

New Brunswick Bio Flo Model C30 bench top chemostats for continuous culture were maintained under aerobic and anaerobic conditions. The media used in aerobic chemostats were either basal salts with nitrogen and glucose or nutrient broth (2 and 4 g/L). Retention time was 7 days; the temperature was maintained at 30°C and the influent nitroguanidine concentration ranged from 75 to 100 ppm (μg/ml).

Anaerobic chemostats were run with nutrient broth (2, 4 and 8 g/L), basal salts, basal salts with glucose, and basal salts with glucose and nitrogen. Initial retention time was 7 days and later dropped to 4 and 2 days. Nitroguanidine influent concentrations ranged from 50 to 100 ppm. Chemostats were operated continuously for up to 3 months at 37°C.

Aerobic cultures were inoculated with activated sludge from the Marlboro Easterly sewage treatment plant (Marlboro, MA) and anaerobic cultures with digest from the Nut Island sewage treatment plant (Boston, MA).

High Performance Liquid Chromatography. Nitroguanidine and nitrosoguanidine were determined on a duPont 830 Liquid Chromatograph with a Perkin Elmer LC55 variable wavelength detector at 263 nm and a Columbia Scientific Supergrator-2

programmable computing integrator. The mobile phase was methanol/water (10/90). Culture media samples were clarified by centrifugation for 10 min at 12,000 rpm and filtered through 0.2 μm filters. Injections of 10 μl were made onto a 25-cm x 4.6-mm duPont Zorbax ODS reverse phase column at 35°C and 1200 psi (8.273 x 10^3 kPa). Retention times were 2.8 min and 2.5 min, and detection limits were 100 ppb (ng/ml) and 500 ppb for nitroguanidine and nitrosoguanidine, respectively.

Mutagenicity Testing. The Ames screening test for mutagenicity was performed with nitroguanidine and nitrosoguanidine according to standard procedures.[4,5] Five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) were used to test concentrations from 5 μg to 5000 μg of each compound per plate with and without metabolic activation.

Chemicals. Nitroguanidine was obtained from Radford Army Ammunition Plant, Radford, VA and recrystallized. Nitrosoguanidine was prepared according to Davis and Rosenquist.[6] Ammeline was prepared according to Cloak and ammelide according to Diebner.[7,8] Aminoguanidine, cyanamide, cyanoguanidine, cyanuric acid, guanidine hydrochloride, and melamine were purchased from Eastman Kodak Co., Rochester, NY. Hydrazine dihydrochloride and urea were purchased from Fisher Scientific Co., Medford, MA.

[4] Ames, B. N. 1979. Supplement to the Methods Paper. University Calif., Berkeley. 1-10.

[5] Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for Detecting Carcinogens and Mutagens with the *Salmonella* Mammalian-Microsome Mutagenicity Test. Mut. Res. 31: 347-364.

[6] Davis, T. L. and E. N. Rosenquist. 1937. Studies in the Urea Series. XV. Transformations of Nitrosoguanidine. Alkylnitrosoguanidines. N-R, N'-R'-Dialkylguanidines. J. Am. Chem. Soc. 15: 2112-2115.

[7] Cloak, L. H. 1961. Production of Ammeline. Brit. Patent No. 869,306.

[8] Diebner, R. L. 1975. Preparation of Ammelides. U.S. Patent No. 3,860,594.

Isolation of Nitrosoguanidine. Nitrosoguanidine was isolated from 4 liters of spent media from an anaerobic chemostat which had been operated at a 7-day retention time with an influent containing 4 g/L nutrient broth and 75 ppm nitroguanidine. No nitroguanidine was detectable in the spent media and nitrosoguanidine was present at 25 ppm. The media were centrifuged at 8,000 rpm for 10 min, filtered through a 0.45-μm filter and evaporated to dryness at 50°C on a rotary evaporator at 15 mm (1.999×10^3Pa) Hg. The media concentrate was resuspended in warm methanol and used for analysis by TLC, HPLC and GC/MS. TLC was performed using ethanol/water (50/50) as developing solvent on cellulose-coated plates with fluorescent indicator. Sport were visualized under UV light and with iodine vapors.

Isolation of Cyanamide, Cyanoguanidine, Guanidine, and Urea. Three liters of spent media from anaerobic chemostat cultures were centrifuged, filtered, and roto-evaporated as before. The concentrate was extracted with warm methanol and cochromatographed with standards on cellulose TLC media. The chromatograms were developed with n-butanol/ethyl acetate/water (4/1/1) and visualized with alkaline ferricyanide-nitroprusside spray.[9] The media concentrate was also extracted with ammonium hydroxide and chromatographed in the same manner. Unknown spots of interest were scraped off TLC plates, eluted, and their identities confirmed by GC/MS.

Other attempts were made to detect urea as the xanthydrol derivative.[10]

[9] Milks, J. E. and R. H. Janes. 1956. Separation and Detection of Cyanamide and its Derivatives and Determination of Urea by Paper Chromatography. Anal. Biochem. 28: 846-849.

[10] Korn, E. D. 1957. Purines and Pyrimidines, P. 631-632. In S. P. Colowick and N. O. Kaplan (eds.), Methods in Enzymology IV. Academic Press, New York.

Isolation of Melamine, Ammeline, Ammelide, and Cyanuric Acid. Concentrates from spent nitroguanidine cultures were prepared as before. The residues were extracted with water, methanol, ammonium hydroxide, or formic acid in different experiments. The extracts were subjected to TLC on cellulose media without fluorescent indicator. The chromatograms were developed with 3N ammonium hydroxide/methanol (60/75).[11] Visualization was carried out with silver nitrate spray followed by heating at 100°C and with p-dimethylamino benzaldehyde.[12] Media extracts were cleaned by passage through C-18 SEP-PAK cartridges (Water's Associates , Milford, MA). Water and methanol washes were chromatographed on cellulose media which were also developed in n-butanol/ethyl acetate/water (4/1/1). Standards were chromatographed with unknowns and appropriate spots from the unknowns were scraped off and eluted for GC/MS analysis.

Detection of Aminoguanidine. Nitroguanidine culture media were examined for the presence of aminoguanidine by HPLC which was performed as with nitroguanidine. The following parameters were used: detection at 210 nm, pressure at 1000 psi (6.894×10^3 kPa), solvent composition at (15/85) methanol/water and the column at room temperature. The retention time for aminoguanidine was 2.5 min, and it emerged just ahead of nitrosoguanidine and nitroguanidine. The limit of detection was 500 ppb.

[11] Knappe, E. and I. Rohdewald. 1966. Identiflzierung von Stickstoffderivaten der Kohlensäure und Untersuchung ihrer Hydrolysate mittels Dünnschichtchro matographie. Zeitschrift fur Analytisches Chemie. 223: 174-181.

[12] See footnote 9, p. 7.

Attempted Isolation of Aminoguanidine as the Salicylidene Derivative. A 1.5-liter spent culture sample originally incubated with nitroguanidine was clarified by centrifugation, basified with 6 N sodium hydroxide and distilled to about one-quarter of its volume. The distillate was collected in 6 N hydrochloric acid. It was evaporated on a rotary evaporator at 55°C, 15 mm (1.999 x 10^3 Pa) Hg to give a semi-solid residue. The residue was extracted with 20 ml of refluxing methanol and allowed to cool one-half hour. The supernatant liquid was decanted from the solid residue and evaporated to dryness on a hot block at 60°C under a nitrogen stream. The extraction was repeated and the extract again evaporated to dryness. The residue was treated with 1.5 ml of 1 N sodium hydroxide and 30 µl of salicylaldehyde. After a few minutes, the reaction mixture was neutralized and extracted with an equal volume of benzene. An authentic sample of aminoguanidine nitrate was derivatized with salicylaldehyde in the same manner as the nitroguanidine culture extract. The benzene extracts from this reaction and the derivative from the culture were cochromatographed on Eastman silica gel TLC medium with fluorescent indicator using 2-propanol as developing solvent. Visualization of the chromatogram by a UV lamp demonstrated that no detectable amount of aminoguanidine was present.

Attempted Isolation of Hydrazine as the Salicylidene Derivative. The derivatized culture extracts obtained in the preceeding section were cochromatographed with salicylazine in the same manner using benzene as developing solvent. Absence of a spot corresponding to salicylazine showed that a detectable quantity of hydrazine was not present in the culture medium.

Another 1.5 L portion of the clarified culture medium was derivatized *in situ* by addition of 0.25 g of salicylaldehyde stirring overnight at room temperature and extraction with benzene. Neither aminoguanidine nor hydrazine could be detected by cochromatography with the salicylaldehyde derivatives as

before. No salicylaldehyde derivative from hydrazine or aminoguanidine could be detected by GC/MS in any of the extracts.

UV Sensitivity of Nitrosoguanidine and Nitroguanidine. Solutions of nitrosoguanidine and nitroguanidine in distilled water, 100 ppm in concentration and 2.5 cm deep, were stirred in beakers under a germicidal UV lamp (Sylvania G15T8). Samples were withdrawn periodically and nitroguanidine and nitrosoguanidine were determined by HPLC.

Nitrosoguanidine was also irradiated *in situ* on cellulose TLC media (with fluorescent indicator). The media were exposed until the nitrosoguanidine had partially disappeared (10 minutes). The chromatograms were developed in n-butanol/ethyl acetate/water (4/1/1) and visualized with alkaline ferricyanide or ninhydrin sprays.

RESULTS

During the first three weeks of incubation there was no significant change in nitroguanidine levels in anaerobic continuous cultures in 4 g/L nutrient broth. Even when periods of incubation extended over three months mixed cultures developed only limited ability to decompose nitroguanidine. Significantly enhanced rates of disappearance of nitroguanidine were detected in both continuous cultures (Fig. 1) and batch cultures (Fig. 2) after repeated subculturing for new anaerobic incubations using the adapted cells. Figure 1 shows the rate of biotransformation of nitroguanidine (initial concentration 75 ppm) in anaerobic continuous culture (4 g/L nutrient broth at a 7-day retention time). Within seven days no nitroguanidine was detected in the culture vessel, and within twelve days there was none in the product reservoir. During this time nitrosoguanidine accumulated in the media. The concentration of nitrosoguanidine rose to 60 ppm in the culture vessel by day 7 and leveled off between 25 and 40 ppm

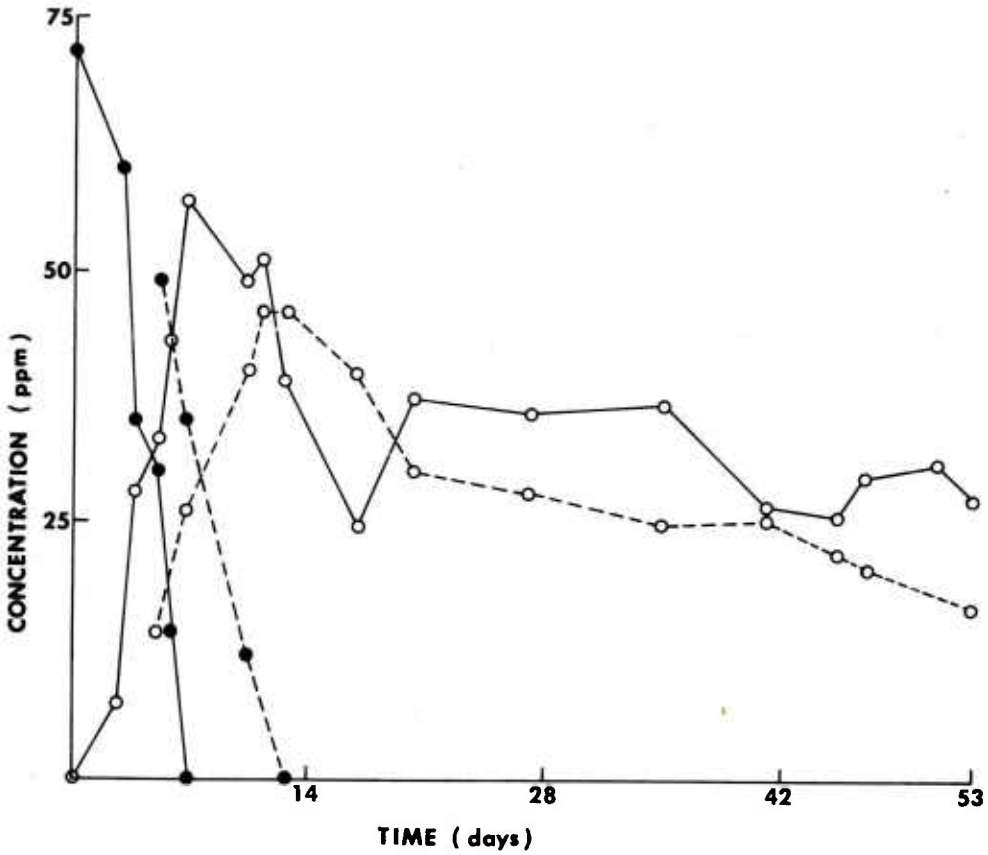


Figure 1. Biotransformation of nitroguanidine (●) to nitrosoguanidine (O) in the culture vessel (——) and product reservoir (----) under anaerobic conditions in chemostat continuous culture.

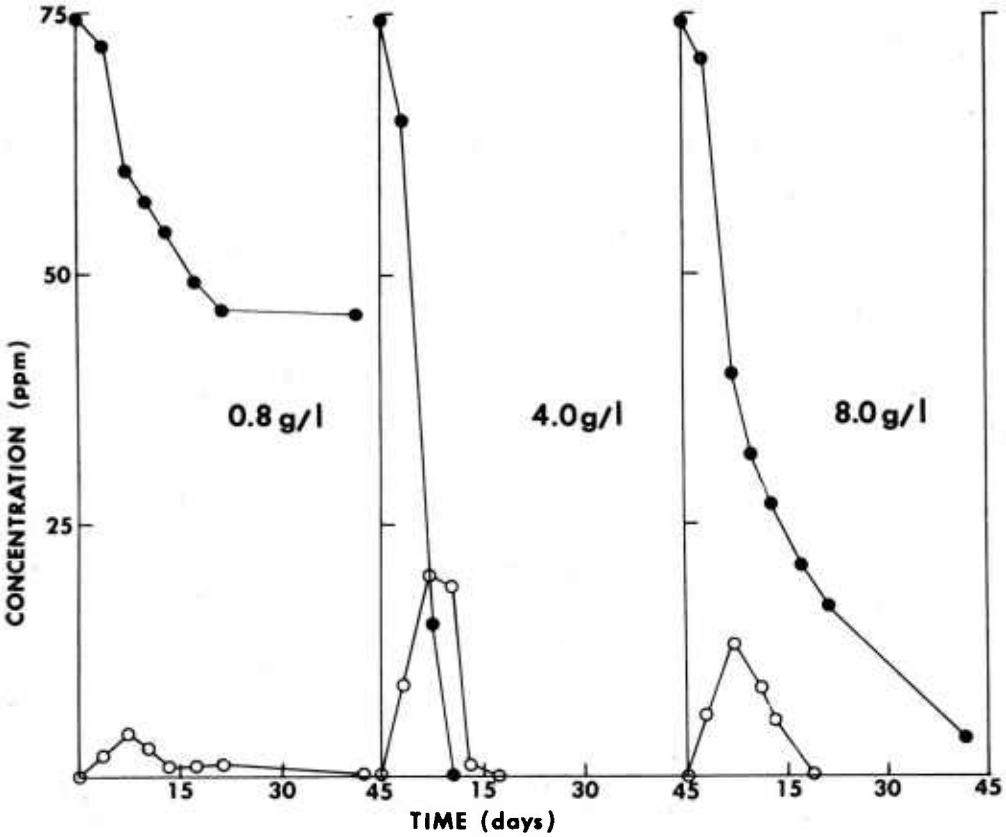


Figure 2. Biotransformation of nitroguanidine (●) to nitrosoguanidine (○) under anaerobic batch conditions in nutrient broth of different strengths.

over a 53-day period. In the spent medium (product reservoir) the concentration of nitrosoguanidine rose to a maximum of 45 ppm by day 12, and then decreased slowly to slightly below the concentration in the culture vessel. Extended periods of continuous culture did not result in lower levels of nitrosoguanidine. Modification of a number of conditions did not further accelerate the rate of disappearance of nitroguanidine or nitrosoguanidine. The concentration of nutrient broth in the culture media was changed to 8 g/L or 2 g/L. Basal salts, basal salts with nitrogen, and basal salts with nitrogen and glucose were used, and the retention times were changed to 2 or 4 days. These alterations only resulted in slower rates of disappearance of both compounds or no decomposition at all in cultures without nutrient broth. Under no conditions did the nitrosoguanidine level fall below about 20 ppm in the culture vessel.

Complete disappearance of nitroguanidine and nitrosoguanidine in batch cultures after subculturing could be achieved, but only in a narrow range of media composition (Fig. 2). In 4 g/L nutrient broth both compounds had completely disappeared by day 17 while in 0.8 g/L and 8 g/L nutrient broth incomplete degradation of nitroguanidine took place even with acclimated cultures.

Under aerobic conditions no decomposition of nitroguanidine was observed in either batch or continuous cultures even after many subcultures for adaptation.

The identity of the nitrosoguanidine isolated from anaerobic continuous cultures was established by comparative TLC and HPLC with a standard sample synthesized by a known method. The mass spectrum of nitrosoguanidine using the probe did not provide unequivocal confirmation of structure due to the production of a large number of low MW fragments.

Cyanamide, cyanoguanidine, and guanidine were identified in extracts of culture media concentrates both by TLC and GC/MS. With the alkaline ferricyan-

ide-nitroprusside spray, cyanamide (R_f = 0.78) and cyanoguanidine (R_f = 0.53) were purple, and guanidine (R_f = 0.26) was orange. No urea (R_f = 0.35 and red/orange) was detected by TLC nor could it be isolated as a xanthydrol derivative from extracts.

Melamine was identified in culture extracts by TLC and GC/MS, but no ammeline, ammelide, nor cyanuric acid were detected. In n-butanol/ethyl acetate/water (4/1/1) melamine had an R_f = 0.38 and appeared yellow after spraying with p-dimethlamino benzaldehyde. In ammonium hydroxide/methanol (60/75) the R_f = 0.78.

No aminoguanidine was detected by GC/MS, HPLC or as a salicylaldehyde derivative by TLC and GC. No hydrazine was detected as the salicylaldehyde derivative by TLC or GC.

Figure 3 illustrates the rates of disappearance of aqueous nitroguanidine and nitrosoguanidine under a short wave UV light source. The rate of disappearance of nitrosoguanidine (slope = -1.48) was twice that of nitroguanidine (slope = -0.74).

TLC of UV photolysis products indicated that cyanamide was formed as nitrosoguanidine decomposed. No evidence of urea or guanidine was found, and the reaction products did not react with ninhydrin. Apparently the photolytic anc chemical pathways for the decomposition of nitrosoguanidine result in similar products.

DISCUSSION

Nitroguanidine did not biodegrade until the anaerobic cultures had a sufficient period for acclimation. Once acclimated, anaerobic cultures readily reduced nitroguanidine cometabolically. This transformation did not occur under aerobic conditions. No evidence was found for further microbiological

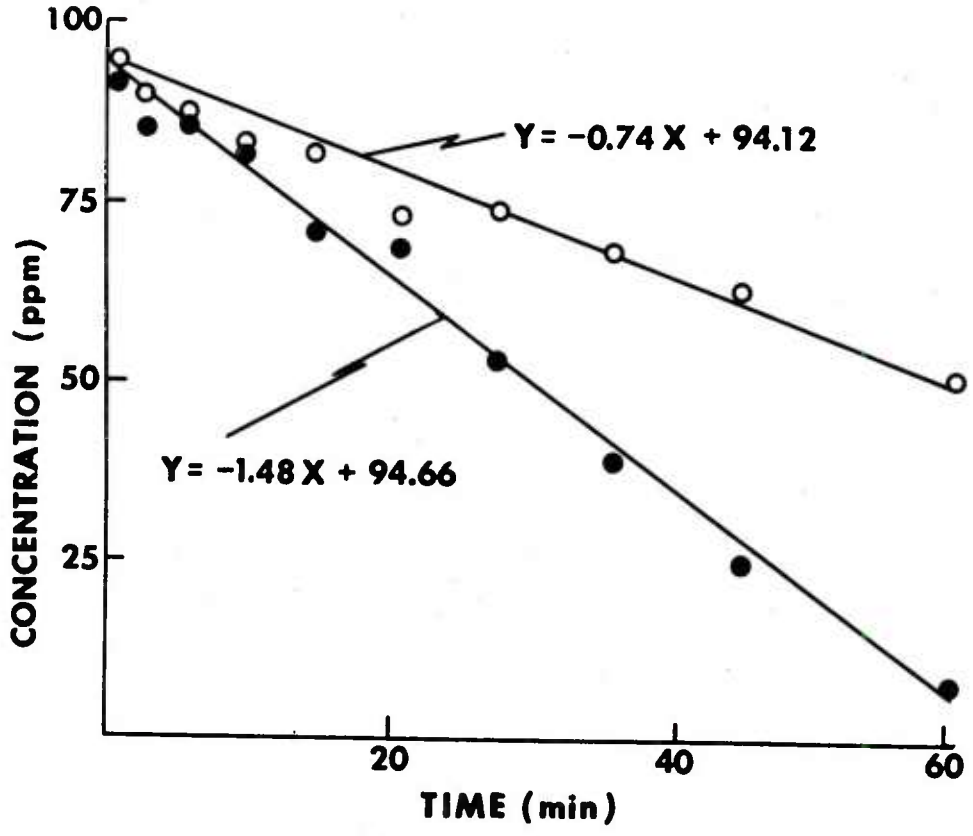


Figure 3. Sensitivity of nitroguanidine (O) and nitrosoguanidine (●) to short wave UV light.

reduction of nitrosoguanidine. Unlike nitroguanidine, we were unable to acclimate cells to completely metabolize nitrosoguanidine by further reduction. Probable reduction products would be hydrazine and urea or aminoguanidine and guanidine. Hydrazine, urea, and aminoguanidine were never detected during these studies and guanidine, although present in trace amounts, can be postulated to arise by an alternate pathway.

It is probable that under these conditions nitrosoguanidine decomposes nonbiologically. Significant quantities of cyanamide, cyanoguanidine, and melamine were identified in the culture effluents. These compounds were reported to be among the products of the chemical decomposition of nitrosoguanidine.[13] The most likely sequence of chemical/physical reactions can be diagrammed in Figure 4. Nitrosoguanidine decomposes to cyanamide and nitrosamide. The cyanamide polymerizes to its dimer cyanoguanidine and finally to its trimer, cyclic melamine. Cyanamide can also react with ammonia to form guanidine. The nitrosamide formed is transitory and decomposes to nitrogen gas and water. Under the conditions prevailing in these experiments, these reactions apparently proceed relatively slowly as all intermediates with the exceptions of the unstable nitrosamide were successfully purified and identified by TLC and GC/MS.

Davis and Rosenquist reported that nitrosoguanidine reacts chemically with ammonia to give cyanamide and nitrosamide.[14] With excess ammonia this reaction proceeds to guanidine, melamine, ammeline, ammelide and a trace of urea. Presumably, under the milder conditions of the present biological studies, the hydroxylated analogs of melamine are not formed.

[13] See footnote 6, p. 6.

[14] Ibid.

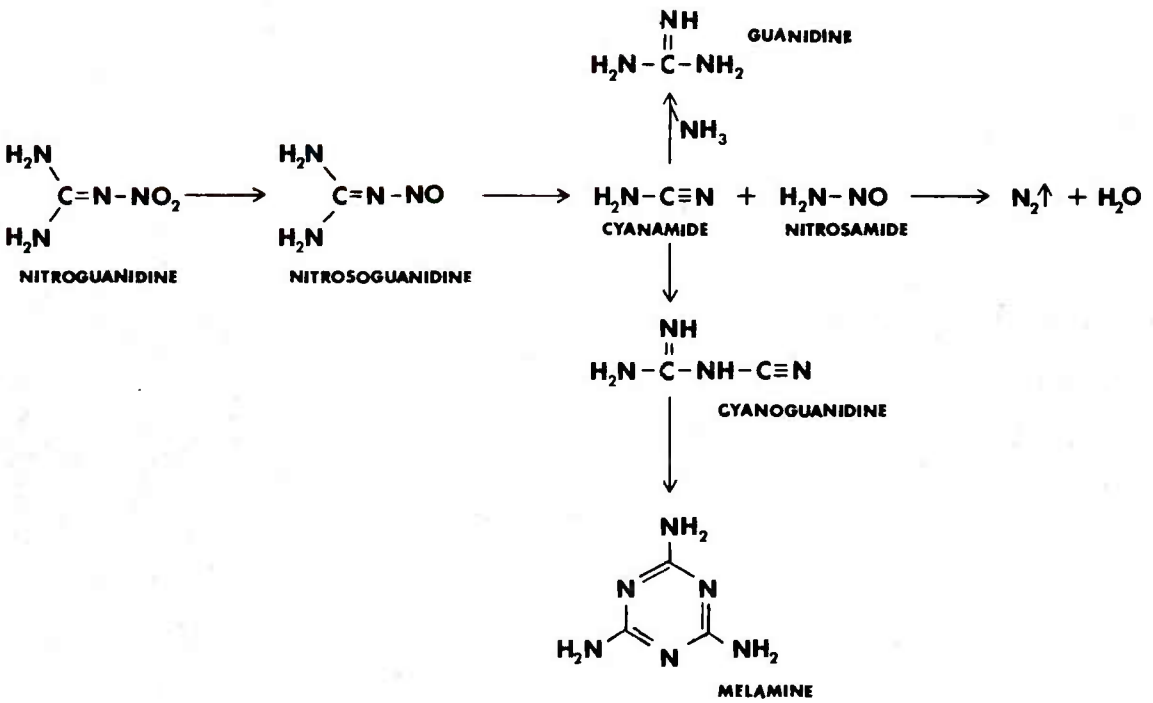


Figure 4. Scheme for chemical and biological degradation of nitroguanidine.

Nitroguanidine and nitrosoguanidine gave negative results in the Ames screening test for mutagenicity. Nitroguanidine however, was reported to be a carcinogen in screening tests with Chinese hamster cells for detection of chromosomal aberrations.[15] Cyanamide is metabolized by plants to arginine through various gaunidino compounds.[16] Calcium cyanamide is used as a plant fertilizer and root stimulator and is not carcinogenic.[17,18] Cyanamide is bacteriostatic at 1000 ppm and toxic to mammals.[19] Melamine presents a low toxicity hazard.[20]

Results to date suggest that nitroguanidine can not be successfully metabolized by microbiological activity to completely innocuous products. Nitrosoguanidine, a nitrosamine, is the primary reduction product, and no evidence was found for further microbiological action on the compound. Chemical decomposition of nitrosoguanidine under conditions suitable for microbiological studies results in the formation of a number of products which pose no significant environmental problems other than mammalian toxicity of cyanamide. At

[15] Ishidate, M. and S. Odashima. 1977. Chromosome Tests with 134 Compounds on Chinese Hamster cells in vitro - A screening for Chemical Carcinogens. Mut. Res. 48: 337-354.

[16] Wuensch, A. and A. Amberger. 1974. Occurance of Arginine in the Metabolism of Plants Fed with Cyanamide. A. Pflanzenphysiol. 72: 359-366.

[17] Iwasaki, K. and R. J. Weaver. 1977. Effects of Chilling, Calcium Cyanamice, and Bud Scale Removal on Bud Break, Rooting and Inhibitor Content of Buds of 'Zinfandel' Grape (*Vitis vinifera* L.). J. Am. Soc. Hortic. Sci. 102: 584-587.

[18] National Cancer Institute. 1979. Bioassay of Calcium Cyanamide for Possible Carcinogenicity. Nat. Tech. Inf. Ser. PB-293625. 113 pp.

[19] Iskandarov, T. I. 1970. Hygienic Standardization of Unbound Cyanamide in Reservoir Waters. Gig. Primen. Toksikol. Pestits. Klin. Otravlenii. 8: 337-340.

[20] Sax, N. I. 1968. General Chemicals, p. 893. In Dangerous Properties of Industrial Materials. Van Nostrand Reinhold Co., New York.

the same time residual levels of nitrosoguanidine remain because of the slow rate of the chemical reactions under these conditions. More stringent chemical conditions would presumably enhance the rate of decomposition of nitrosoguanidine and change the number and the types of products as documented by Davis and Rosenquist.[21]

The demonstrated sensitivity of both nitroguanidine and nitrosoguanidine to UV light suggests this treatment as an alternative in alleviating pollution hazards associated with nitroguanidine laden waste streams.

CONCLUSIONS

Nitroguanidine is cometabolically reduced to nitrosoguanidine under anaerobic conditions. Nitrosoguanidine decomposed nonbiologically to cyanamide, cyanoguanidine, melamine, and guanidine. No evidence was found for urea, aminoguanidine, hydrazine, ammeline, ammelide, or cyanuric acid in culture extracts. No biotransformation of nitroguanidine occurred under aerobic conditions. Nitroguanidine and nitrosoguanidine test negative in the Ames test for mutagenicity and both compounds are sensitive to UV light.

[21] See footnote 6, p. 6.

REFERENCES

Ames, B. N. 1979. Supplement to the Methods Paper. University Calif., Berkeley. 1-10.

Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for Detecting Carcinogens and Mutagens with the *Salmonella* Mammalian-Microsome Mutagenicity Test. Mut. Res. 31: 347-364.

Bissett, F. H. and L. A. Levasseur. 1976. Analytical Methods for Nitroguanidine and Characterization of its Degradation Products. Technical Report TR-76/47. U.S. Army Natick Research and Development Command, Natick, MA.

Cloak, L. H. 1961. Production of Ammeline. Brit. Patent No. 869,306.

Davis, T. L. and E. N. Rosenquist. 1937. Studies in the Urea Series. XV. Transformations of Nitrosoguanidine. Alkylnitrosoguanidines. N-R, N'-R'-Dialkylguanidines. J. Am. Chem. Soc. 15: 2112-2115.

Diebner, R. L. 1975. Preparation of Ammelides. U.S. Patent No. 3,860,594.

Ishidate, M. and S. Odashima. 1977. Chromosome Tests with 134 Compounds on Chinese Hamster cells in vitro - A screening for Chemical Carcinogens. Mut. Res. 48: 337-354.

Iskandarov, T. I. 1970. Hygienic Standardization of Unbound Cyanamide in Reservoir Waters. Gig. Primen. Toksikol. Pestits. Klin. Otravlenii. 8: 337-340.

Iwasaki, K. and R. J. Weaver. 1977. Effects of Chilling, Calcium Cyanamide, and Bud Scale Removal on Bud Break, Rooting, and Inhibitor Content of Buds of 'Zinfandel' Grape (*Vitis vinifera* L.). J. Am. Soc. Hortic. Sci. 102: 584-587.

Kemula, W., M. K. Kalinowski, T. M. Krygowski, J. A. Lewandowski, and A. J. Walasek. 1970. Investigation of N-Nitroderivatives. Equilibria of Nitrourea and Nitroguanidine in Aqueous Solutions. Bull. Acad. Pol. Sci., Chem. Ser., 18: 445-461.

Knappe, E. and I. Rohdewald. 1966. Identiflzierung von Stickstoffderivaten der Kohlensäure und Untersuchung ihrer Hydrolysate mittels Dünnschichtchromatographie. Zeitschrift fur Analytisches Chemie. 223: 174-181.

Korn, E. D. 1957. Purines and Pyrimidines, p. 631-632. In S. P. Colowick and N. O. Kaplan (eds.), Methods in Enzymology IV. Academic Press, New York.

Milks, J. E. and R. H. Janes. 1956. Separation and Detection of Cyanamide and its Derivatives and Determination of Urea by Paper Chromatography. Anal. Biochem. 28: 846-849.

National Cancer Institute. 1979. Bioassay of Calcium Cyanamide for Possible Carcinogenicity. Nat. Tech. Inf. Ser. PB-293625. 113 pp.

Small, M. J. and D. H. Rosenblatt. 1974. Munitions Production Products of Potential Concern as Waterborne Pollutants - Phase II. U.S. Army Bioengineering Research and Development Laboratory, Aberdeen Proving Ground, MD. Tech. Rpt. 7404.

Sax, N. I. 1968. General Chemicals, p. 893. In Dangerous Properties of Industrial Materials. Van Nostrand Reinhold Co., New York.

Wuensch, A. and A. Amberger. 1974. Occurance of Arginine in the Metabolism of Plants Fed with Cyanamide. A. Pflanzenphysiol. 72: 359-366.