A DATA BASE ASSESSMENT OF ENVIRONMENTAL FATE ASPECTS
OF NITROGUANIDINE

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Nitroguanidine is scheduled for production at Sunflower Army Ammunition Plant, DeSoto, KS. Sufficient gaps exist in the data available on the environmental fate and aquatic and mammalian toxicity of nitroguanidine to warrant further studies.
Data describing physical transport of nitroguanidine are totally lacking. Although solubility of nitroguanidine is well known, no experimental information is available for the octanol–water partition coefficient (estimated $K_{ow} = 2.7$) and bioconcentration in aquatic species. Hydrolisis rates of nitroguanidine in natural water are not known. Photolysis in natural waters where humics and suspended solids are present must be measured. Other chemical transformations, including oxidation/reduction, will have to be screened and measured. Nitroguanidine is capable of being anaerobically biodegraded; however, whether or not microorganisms indigenous to the creeks receiving nitroguanidine effluents can also degrade the compound, and if so, at what rate, needs to be determined. It is not known if water plants will take up nitroguanidine. More data are required to precisely assess the hazard of nitroguanidine to mammalian and aquatic species.
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INTRODUCTION

A part of the mission of the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) is the evaluation of the environmental and health hazard of military-unique pollutants generated by US Army munitions manufacturing facilities. The purpose of these evaluations is the recommendation of water quality criteria for promulgation by The Surgeon General.

As a first step in the generation of a data base, existing information about a given pollutant is sought. The objective of this report is to assess the data base available on nitroguanidine and to recommend studies needed to fill the gaps found. This assessment follows those completed in the past\textsuperscript{1,2} and essentially is concerned with more current information.

Nitroguanidine is a component of US Army triple-base propellant mixtures. Its presence in the mixtures, which also contain nitrocellulose and nitroglycerin, provides thrust and stability while reducing the burning temperature and flash intensity.\textsuperscript{3}

Nitroguanidine is scheduled to be manufactured at Sunflower Army Ammunition Plant (SAAP), DeSoto, Kansas, located in northeast Kansas (see map, Fig. 1) near the Kansas River. SAAP nitroguanidine production facilities are new and in the final prove-out stage. Wastewaters generated by the nitroguanidine facility were originally intended to be pollutant-free; the anticipated discharge was to contain only evaporation and vacuum crystallization condensates, cooling tower blowdown, and wash water from water softeners.\textsuperscript{4} However, prove-out operations have shown wastewaters to be contaminated.\textsuperscript{4} This contaminated wastewater now flows into lagoons and holding ponds; any leakages, leachates, or overflow (SAAP has limited storage capacity) will exit to the environment.

OBJECTIVE

The objective of this report is to provide a current assessment of the data base on nitroguanidine, including environmental fate, mammalian and aquatic toxicity, and state-of-the-art analytical methods.

APPROACH

Each element of the stated objective was employed in a computer literature search as descriptors of nitroguanidine. Table 1 lists the data bases searched.
Figure 1. SAAP Installation Map.
Other literature reviewed included US Army final reports, Technical Reports, etc., from contracts and in-house surveys. Specific information sought:

* Solubility in Water - Basic to all studies, required for octanol-water partition coefficient and sediment sorption estimations and measurements.

* Vapor Pressure - To determine volatility of a chemical from water.

* Sediment Sorption - Indicates capability of a compound to bind to sediment.

* Photolysis - Measures the stability of a chemical exposed to sunlight.

* Octanol-Water Partition Coefficient - Measures tendency of a chemical to reside in a lipid versus a water phase and is correlated with the bioconcentration factor for aquatic species.

* Oxidation/Reduction - Measures loss of chemical through environmental oxidative or reductive chemical processes.

* Hydrolysis - Measures loss of a chemical due to splitting of chemical bonds by water.

* Biodegradation - Measures loss of chemical through degradation or transformation by microorganisms, aerobically or anaerobically.

* Bioadsorption - Necessary to determine if chemical is non-biologically bound to microorganisms.

* Biouptake - Measures loss of a chemical from water due to uptake by plants, which may accumulate or transform the chemical.
Bioaccumulation - Measures accumulation of a chemical within tissues of an organism, where concentration of the chemical may be orders of magnitude above the surrounding milieu.

Aquatic Toxicity - Determines if a chemical is a hazard to aquatic species and at what levels.

Mammalian Toxicity - Defines toxicity of a chemical to mammalian species with the purpose of providing safe exposure levels for humans.

RESULTS

PHYSICAL-CHEMICAL PROPERTIES

Nitroguanidine chemistry has been the subject of several reviews. Small and Rosenblatt,\(^1\) in 1974, reviewed much of the chemistry of nitroguanidine production while, a year later, Burrows and Dacre\(^2\) reviewed the chemistry and aquatic toxicity.

Nitroguanidine exists in two tautomeric forms, as shown below.

\[
\text{Form A} \quad \begin{array}{c}
\text{NH}_2 \\
\text{C-N-N} \\
\text{NH}_2
\end{array}
\quad \text{Form B} \quad \begin{array}{c}
\text{NH}_2 \\
\text{C-NH-NO}_2 \\
\text{NH}
\end{array}
\]

Form A "predominates in acidic, neutral, and slightly basic media".\(^5\) Aqueous solutions of nitroguanidine subjected to ultraviolet light exhibit one band over the pH range of 2-12. The \(\lambda_{\text{max}}\) at the band is 264 nm (\(E_{\text{max}} = 13,000\)).\(^6\) As conditions become increasingly alkaline, a new band shows up at 246 nm. Bissett and Levasseur state that the band shift is the evidence of the appearance of Form B.\(^6\) Table 2 shows the physical-chemical properties of nitroguanidine.
**TABLE 2. PHYSICAL-CHEMICAL PROPERTIES OF NITROGUANIDINE**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Molecular Formula</td>
<td>NH₂NH₂C(=N)NO₂</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>104.074</td>
</tr>
<tr>
<td>Appearance</td>
<td>Colorless crystals</td>
</tr>
<tr>
<td>Melting Point</td>
<td>Decomposes at 232°C</td>
</tr>
<tr>
<td>Density</td>
<td>1.72 g/cm³</td>
</tr>
<tr>
<td>Vapor Pressure (mmHg)</td>
<td>Extremely low</td>
</tr>
<tr>
<td>Heat of Combustion</td>
<td>209 kcal/mole</td>
</tr>
<tr>
<td>Stability</td>
<td>Sensitive to ultraviolet light, absorbs at 264 nm Explosive when shocked or exposed to heat or flame</td>
</tr>
<tr>
<td>Solubilities</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>4.4 g/L at 25°C</td>
</tr>
<tr>
<td></td>
<td>83.5 g/L at 100°C</td>
</tr>
<tr>
<td>Base (1N KOH)</td>
<td>12 g/L at 25°C</td>
</tr>
<tr>
<td>Acid (40%H₂SO₄)</td>
<td>80 g/L at 25°C</td>
</tr>
</tbody>
</table>


**PRODUCTION**

The manufacture of nitroguanidine at SAAP is a new procedure with the following sequence:

1) Calcium carbide → converted → Calcium cyanamide

2) Calcium cyanamide + NH₄NO₃ → Guanidine nitrate + NH₃

3) Guanidine nitrate + H₂SO₄ → Nitroguanidine

The new nitroguanidine production facility at SAAP is designed to produce 40 tons per day, generating wastewaters from SAAP equal to as much as 1,075,680 gpd.

**SITE DESCRIPTION**

Sunflower AAP and adjacent areas have been subjected to several surveys covering native species and natural water quality. Creeks in the area, including Spoon, Kill, Captains, and Hansen Creeks, are for the most part well-buffered, hard waters and, at the time of the surveys, in good condition. Sewage outfalls and agricultural run-off from the plant and neighboring areas contribute to the high nutrient load of these creeks. Wastewater from...
the nitroguanidine pilot facility presently exits the plant via a series of lagoons and ponds to Hansen Creek, which also receives effluents from Clearwater Village sewage treatment plant. Hansen Creek joins Kill Creek downstream, and these waters are ultimately joined to the Kansas River (see Fig. 1).

A survey was conducted by the US Army Environmental Hygiene Agency in February 1982. The purpose of the survey was to characterize "wastewaters associated with the manufacture of nitroguanidine at Sunflower AAP." Samples from the following sites were analyzed: nitroguanidine pilot plant outfall, Kill Creek, upper lagoon, lower lagoon, concrete basin raw water treatment plant, pilot plant evaporator condensate, pilot plant crystallizer, Pond A, and Pond B. At all sites, except at the pilot plant crystallizer, significant quantities of nitroguanidine and guanidine nitrate were found. The conclusion from these analyses was that "significant levels of contamination" exist for nitroguanidine and guanidine nitrate in all but one of the test sites.

ANALYTICAL METHODS

Prior to the advent of modern automated analytical techniques, several wet chemistry methods existed for the detection and measurement of nitroguanidine. In 1976 Bissett and Levasseur studied column materials and solvent systems for high pressure liquid chromatography (HPLC) of nitroguanidine. In these experiments the test samples consisted of nitroguanidine plus TNT, RDX, and HMX. By employing a &-porasil column and 25 percent isopropanol/75 percent hexane or 10 percent isopropanol/90 percent CHCl₃, the authors were able to separate the components and detect nitroguanidine. Table 3, from Bissett and Levasseur, shows the relative retention times for the components of the test mixture.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>α-TNT</th>
<th>RDX</th>
<th>HMX</th>
<th>Nitroguanidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropyl/Hex</td>
<td>1.4</td>
<td>3.5</td>
<td>8.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Isopropyl/CHCl₃</td>
<td>1.2</td>
<td>2.3</td>
<td>6.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

From Bissett & Levasseur, 1976.

Recent work by Kaplan et al. at US Army Natick Laboratories, produced an HPLC analysis of microbiological culture media (clarified). Table 4 shows test conditions and detection limit data for nitroguanidine and nitrosoguanidine.

This method will undoubtedly prove satisfactory for the analysis of nitroguanidine in wastewaters.
TABLE 4. HPLC ANALYSIS OF NITROGUANIDINE AND
NITROSOGUANIDINE IN CULTURE MEDIA

<table>
<thead>
<tr>
<th>Instrument</th>
<th>DuPont 830</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>Variable Wavelength (Perkin-Elmer LC55) at 263 nm</td>
</tr>
<tr>
<td>Solvent</td>
<td>Methanol/Water (10/90)</td>
</tr>
<tr>
<td>Column</td>
<td>25cm x 4.6mm ODS reverse-phase (DuPont Zorbax)</td>
</tr>
<tr>
<td>Temperature</td>
<td>35°C</td>
</tr>
<tr>
<td>Pressure</td>
<td>$8.273 \times 10^3$ kPa</td>
</tr>
<tr>
<td>Retention Time</td>
<td>Nitroguanidine: 2.8 min Nitrosoguanidine: 2.5 min</td>
</tr>
<tr>
<td>Detection Limits</td>
<td>Nitroguanidine: 100 ng/mL (ppb) Nitrosoguanidine: 500 ng/mL (ppb)</td>
</tr>
</tbody>
</table>

SEDIMENT SORPTION

No data were found in the literature. However, receiving waters at Sunflower AAP have high nutrient loads and suspended solids.7,8 Because of this high organic load, physical sorption will probably not be significant; however, chemical sorption cannot be ruled out. Screening studies are required to determine if sediment sorption of nitroguanidine occurs and is a dominant pathway in the fate of this chemical.

BIOADSORPTION/BIOUPTAKE

No data are available on either topic. Screening studies should reveal the importance of these processes.

SOLUBILITY IN WATER

The solubility of nitroguanidine in water is relatively high compared to most explosives and well known.3 At 25°C the solubility is 4.4 g/L. As temperature increases, the water solubility also increases, so that at 100°C 90 g nitroguanidine will dissolve in 1 L water.

VAPOR PRESSURE

Although very little information was available on the vapor pressure of nitroguanidine, most references estimate that it is very low. Therefore volatilization should not prove to be a significant pathway in an environmental fate determination. However, hard data are required to make an accurate assessment.
OCTANOL-WATER PARTITION COEFFICIENT

No information is available on the octanol-water partition coefficient of nitroguanidine. However, since this compound is readily water soluble, and, since there is an inverse relationship between water solubility and bioconcentration, the significance of this pathway in the environmental fate of nitroguanidine is probably low.

OXIDATION/REDUCTION

No relevant environmental data are available. However, as with other explosives, the nitro group may be capable of reduction in the presence of iron (II). Full screening studies are necessary to assess the importance of these processes in the fate evaluation of nitroguanidine.

HYDROLYSIS

Bissett and Levasseur reported the hydrolysis of nitroguanidine under neutral, acidic, and basic conditions. When heated under flux conditions for 24 hr, an aqueous solution of nitroguanidine exhibited no changes in structure as detected by ultraviolet or infrared spectroscopy and thin-layer or high pressure liquid chromatography.

Under dilute (0.1M, 0.5M, and 1.0M HCl) acid conditions at 100°C for 24 hr, nitroguanidine showed no structural changes. Only strong acid (2500 moles acid to 1 mole nitroguanidine) was capable of degrading nitroguanidine.

Nitroguanidine can be base-hydrolysed at pH 10. When nitroguanidine in 0.5M NaOH (pH 12) is heated to 55°C, hydrolysis occurs in 2 hours. This is shown by the loss of the UV absorption peak at 264 nm. At pH 10.5 in 1M NaOH, 55°C, nitroguanidine is hydrolysed in 4 hours. Products of base hydrolysis, as detected by GC, were N₂O and NH₃ gases.

Bissett and Levasseur also determined the rates of the base hydrolysis of nitroguanidine. The test solution was 6.7 x 10⁻⁵M nitroguanidine at pH 12.6 and 42°C and pH 9.9 at 66°C. Table 5 shows the rates, calculated from the pseudo-first-order plots. Decrease in nitroguanidine was followed by a decrease in UVmax at 265 nm with time at constant temperature and pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>Temperature</th>
<th>Rate K_h</th>
</tr>
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<tbody>
<tr>
<td>12.6</td>
<td>42°C</td>
<td>3.7 x 10⁻³/min</td>
</tr>
<tr>
<td>9.9</td>
<td>66°C</td>
<td>4.6 x 10⁻⁴/min</td>
</tr>
</tbody>
</table>

Linearity of the plots of log (A₀ - Aₜ) versus time disappears at 60°C and >pH 11.5; plots show a downward turn.
Concentration of buffer had no effect on the hydrolysis rate, indicating that the process is subject to specific base catalysis. The Arrhenius activation energy ($E_a$) at pH 11.8 was calculated from a linear plot of the rate data to be 22.5 kcal. As a result of these studies, it is seen that nitroguanidine will be only very slowly degraded by hydrolysis in natural waters.

PHOTOLYSIS

Studies by Bissett and Levasseur on ultraviolet absorption spectra reveal that nitroguanidine in aqueous solutions shows only one band at pH 2-12. The $\lambda_{max}$ of this band is 264 nm ($E_{max} = 13,000$), which decreases under more alkaline conditions as a new band at $\lambda_{max}$ 246 nm appears. Bissett and Levasseur state that this shift in absorption is indicative of a shift from structure (A) of nitroguanidine to structure (B). A recent study by Kaplan et al. (1982) looked at the ultraviolet sensitivity of 100 ppm nitroguanidine and also nitrosoguanidine in distilled H$_2$O, pH 6.0, in containers 2.5 cm deep. Analysis indicated that the rate of disappearance of nitrosoguanidine ($slope = -1.48$) was twice that of nitroguanidine ($slope = -0.74$), and that both compounds are sensitive to shortwave ultraviolet light. The main product of the UV photolysis of nitrosoguanidine detected by TLC (Kaplan et al.) is cyanamide. No photolysis rates were given, and tests were not performed in natural waters, which have been shown to contain humic substances enhancing photolysis of other explosive compounds.

BIODEGRADATION

A first attempt to assess the biodegradation of nitroguanidine was reported by the American Cyanamid Company in 1955. Nitroguanidine was mixed with dry soil (200 g), from 2% to 0.2% of the total soil mass, and then wetted with B.O.D. dilution water. Breakdown of the test compound was ascertained by measuring evolution of CO$_2$ or ammonia. The test was run for 2 weeks. Results were ambiguous due to overlap in CO$_2$ production concentrations between the controls and test samples.

Kaplan (1982) has reported the anaerobic biotransformation of nitroguanidine to nitrosoguanidine. Microorganisms were those of activated sludge. Cultures required acclimatization, and biotransformation was cometabolic, with 4 g/L nutrient broth optimum. Nitrosoguanidine was not found to be further reduced biologically but was reduced abiotically in a degradative pathway leading to cyanamide, cyanoguanidine, melamine, and guanidine.

Because of the high solubility of nitroguanidine, sediment-bound anaerobic microorganisms will probably encounter little nitroguanidine. This remains to be seen. Also, sludge microorganisms are not necessarily similar to the indigenous biota at SAAP; therefore, screening and rate studies will have to be done.

AQUATIC TOXICITY

In the report from the American Cyanamid Company in 1955 referenced by van der Schalie, a flow-through test using fathead minnows was conducted with nitroguanidine at a concentration at 3,650 mg/L. This high concentration
(near the solubility limit for nitroguanidine) killed 3 of 10 fish; however, no toxicant-related deaths occurred at lower concentrations. Other parameters, such as feeding, schooling, and balance behavior, were not affected at any concentration of nitroguanidine tested. Later USAMBRDL studies tested the effects of nitroguanidine at 2000 and 1,175 mg/L with four fish and five invertebrate species. The compound was non-toxic at these levels.

Tests with mixed fresh water algae over 42 days revealed that growth was stimulated at 40 mg/L nitroguanidine but inhibited by >130 mg/L. Since algal populations weren't defined, and the basic testing procedure was less than ideal, these tests do not meet currently accepted standards and should be repeated.

MAMMALIAN TOXICITY

Studies performed at Hazelton Laboratories in 1955 using nitroguanidine included feeding and skin irritation. A single oral dose of 4.64 g/kg nitroguanidine into male albino rats produced no permanent toxic effects. The oral LD₅₀ for rats was reported to be > 4.64 g/kg. Continuous feeding of rats for 30 days on a diet supplemented with 1.0 percent (0.93 g/kg) nitroguanidine produced no observable effects. Upon sacrifice of test animals, no significant gross pathology was found. The same study tested the effects of dermal application of nitroguanidine on albino rabbits. Single applications of a nitroguanidine paste (aqueous), providing a dose level of up to 10 g/kg over 24 hr exposure showed no toxic effects. Neither were there any symptoms of systemic toxicity observed during the week following the test. These data seem to indicate that nitroguanidine is relatively non-toxic to mammalian systems. A literature evaluation sponsored by the USEPA was performed for USAMRDC in 1975. The nitroguanidine toxicity data of the American Cyanamid Company in 1955 were judged to be sound, but incomplete. They recommended the following studies to complete the toxicity assessment of nitroguanidine:

- "Acute LD₅₀ studies in two species and by the oral route of administration."

- "A 90-day subchronic toxicity study in which data on clinical chemistry, hematology, histopathology and cardio-vascular physiology are generated (the American Cyanamid study did not)."

- "A comparative metabolism study in two species with emphasis on absorption, since this may be the reason for low acute toxicity, and on biotransformation at both high and low doses. (Differing metabolic pathways at varying dose levels might suggest different pathological response with low chronic doses than with high acute doses.)"

- "A metabolic study with pregnant animals to determine the degree of absorption by the fetuses. Finding significant levels in the offspring would require reproductive studies in two species."

- "A chronic toxicity study (2-year) would be required only if the data from the above studies suggested the potential for high toxicity with lifetime exposure."
Carcinogenicity/Mutagenicity

Studies performed in 1977\textsuperscript{23} reported nitroguanidine to be a mutagen for Chinese hamster cells in screening tests for chromosomal aberrations. In 1980 McGregor\textsuperscript{24} performed Escherichia coli DNA repair tests by applying 10 mg per plate to a polymerase-deficient strain of E. coli. No "preferential" toxicity was observed. Ames\textsuperscript{25} tests using 10 mg/L nitroguanidine were done with Salmonella typhimurium cultures (strains TA98, 1535, 100, 1537, 1538); no mutagenic activity was observed. Mitotic recombinogenic Activity Tests, using cultures of the yeast Saccharomyces cerevisiae and nitroguanidine at 22.7 mg/mL, with 150 min incubation, did not reveal any recombinogenic activity attributable to nitroguanidine.

In 1982 Kaplan repeated the Ames testing of nitroguanidine but also included nitrosoguanidine. The same strains of S. typhimurium were used, and the chemicals were tested at levels of 5 to 5000 µg/plate with and without metabolic activation. All test results were negative.

Conclusions

Sufficient gaps exist in the data available on the environmental fate and aquatic and mammalian toxicity of nitroguanidine to warrant further studies.

Data describing physical transport of nitroguanidine are totally lacking. Although solubility of nitroguanidine is well-known, no experimental information is available for the octanol-water partition coefficient (estimated $K_{ow} = 2.7$) and bioconcentration in aquatic species. Hydrolysis rates of nitroguanidine in natural water are not known. Photolysis in natural waters where humics and suspended solids are present must be measured. Other chemical transformations, including oxidation/reduction, will have to be screened and measured. Nitroguanidine is capable of being anaerobically biodegraded; however, whether or not microorganisms indigenous to the creeks receiving nitroguanidine effluents can also degrade the compound, and if so, at what rate, needs to be determined. It is not known if water plants will take up nitroguanidine. More data are required to precisely assess the hazard of nitroguanidine to mammalian and aquatic species.

Recommendations

Recommend that environmental fate studies, including physical transport and chemical and biological transformations, be undertaken. Also recommend aquatic and mammalian toxicity studies to include 90-day feeding in rats and mice, and metabolic studies to evaluate the potential hazard of nitroguanidine.
LITERATURE CITED


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