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HEALTH ADVISORY FOR NITROGUANIDINE (NQ)

Criteria and Standards Division
Office of Drinking Water
U.S. Environmental Protection Agency
Washington, DC 20460

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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environmental Safety and Occupational Health (Office of the Assistant Secretary of the Army, Installations and Logistics; OASA, I&L), and the U.S. Environmental Protection Agency (EPA), Office of Drinking Water (ODW), Criteria and Standards Division, for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines and analytical methods, and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, Ten-day, Longer-term, and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water, nor are they legally enforceable standards. They are not issued as official regulations, and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army, on the munitions chemical nitroguanidine (NQ) have been reviewed, and relevant findings are presented in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted for critical internal and external review by the EPA.

A companion document entitled "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for NQ" is included in this report.

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EXECUTIVE SUMMARY

Nitroguanidine (NQ), a colorless, crystalline solid, is used in military munitions formulations. NQ is rapidly absorbed through the gastrointestinal tract, rapidly enters the blood, and is quickly excreted unchanged in the urine. This passage is facilitated by the small molecular size of the compound. The route of administration has not been found to affect the compound's disposition. In rats, 40 to 50% of the [^{14}C]NQ administered orally was found in the urine within 4 hours; approximately 95% of the administered radiolabel was recovered in the urine within 48 hours after dosing. Small amounts of radiolabel found in the feces (0.4-1.6%) were considered to be due to urine contamination. [^{14}C]CO_2 was not detected in expired air following administration of [^{14}C]NQ.

Limited information was found regarding the distribution of NQ. One hour after an oral dose of [^{14}C]NQ, radioactivity concentrated primarily in the gastrointestinal tract but was distributed in the blood to the major organs (e.g., liver, kidneys, heart, lungs, spleen). The kinetics of NQ in the blood following oral or intravenous administration was not dose dependent in rats dosed with 20 or 200 mg/kg [^{14}C] NQ. Within 48 hours after dosing, no significant radioactivity (<0.02% of the administered dose) remained in any major organs. Low NQ tissue levels reflected the rapid removal of the compound from the body. Animal studies suggest that NQ is not extensively metabolized; following oral administration of [^{14}C]NQ to rats, approximately 100% of the administered radioactivity was found in the urine within 48 hours as unchanged NQ.

The health effects of NQ have not been studied in humans. The oral LD_{50} values of NQ in mice and rats are approximately 3.9 and 10.2 g/kg, respectively. After a single dose, effects on respiration, the gastrointestinal tract, and the central nervous system (CNS) were exhibited. Mice were more susceptible to CNS effects; females were most susceptible to convulsions at doses of 6.31, 5.01, and 3.98 g/kg.

In a 14-day study, serum electrolytes were decreased and water consumption was increased in male and female rats fed 1,000 mg/kg/day. Reduced heart weights were also observed in females at the same dose level. No compound-related histopathological effects were seen. No significant changes in electrolytes or organ weights occurred at 316 mg/kg/day. NQ was considered to be an osmotic diuretic and was found to be excreted unchanged in the urine. The Lowest-Observed-Adverse-Effect Level (LOAEL) from this study was 1000 mg/kg/day and the No-Observed-Adverse-Effect Level (NOAEL) was 316 mg/kg/day.

In a 90-day feeding study in rats, NQ was administered in the diet at levels ranging from 100 to 1,000 mg/kg/day. Mean body weights were decreased for 9 of 13 study weeks in females fed NQ in the diet at 1,000 mg/kg/day. Water consumption was increased in males and females receiving the same dose. No compound-related histopathological lesions were observed in dosed rats. The LOAEL was 1000 mg/kg/day and the NOAEL was 316 mg/kg/day based on increased water consumption and decreased electrolytes in male and female rats and reduced heart weights in female rats.

In a 90-day feeding study with mice, reduced heart weights occurred in males fed NQ in the diet at 1,000 mg/kg/day. Water consumption was increased in males and females at the same dose. No compound-related histopathological effects were exhibited, and no remarkable changes in organ weights occurred at 316 mg/kg/day.

In a chronic toxicity study, NQ caused significant changes in hematologic indices and in the enzyme-generating function of the liver at dose levels of 0.05 and 0.5 mg/kg/day. However, results are inconclusive because of the absence of any other reported data. No carcinogenicity studies on NQ are currently available. NQ is classified in Group D: Not Classified as to Human Carcinogenicity.

The <u>in vitro</u> and <u>in vivo</u> genetic toxicology assays conducted with NQ were uniformly negative. With the exception of a single mouse dominant lethal assay, however, the various studies with NQ were flawed by either an inability to demonstrate test material interaction with the target cell

(i.e., cytotoxicity) or the performance of assays under suboptimal conditions.

No reproductive effects were found in rats after dosing with 20 to 500 mg NQ/kg for 40 days; however, results are inconclusive because of the absence of any other reported data. Developmental effects (increased incidence of resorptions, decreased pup size and weight, and increased incidence of skeletal variations) were seen at 1,000 mg NQ/kg/day in rats and rabbits. NQ caused maternal toxicity in rats at 1,000 mg/kg/day but was not teratogenic. There was equivocal evidence for developmental toxicity in rabbits at 100, 316, and 1,000 mg/kg/day.

Based on these findings, the Longer-term Health Advisory (HA) for a 10-kg child is 11 mg/L (11,000 μg/L). In the absence of adequate animal data to determine a One-day Health Advisory, the Ten-day HA for 10-kg child, 11 mg/L (11,000 μg/L), is established as a conservative estimate of the One-day HA. The Longer-term HA for an adult is established at 37 mg/L (37,000 μg/L). A Lifetime HA of 0.74 mg/L (740 μg/L) for an adult is determined based on a Drinking Water Equivalent Level (DWEL) of 4.0 mg/L (4,000 μg/L). The DWEL is based on a Reference Dose (RfD) of 0.105 mg/kg/day where the effect was the absence of body and organ weight changes in female rats fed NQ for 90 days.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Drinking Water (ODW), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State, and local officials responsible for protection of public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards, and they are subject to change as new information becomes available.

Health Advisories are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime), and Lifetime exposures based on data describing noncarcinogenic endpoints of toxicity. Health Advisories do not quantitatively incorporate any potential carcinogenic risk from such exposure. For substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated value. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit, and probit models. There is no current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

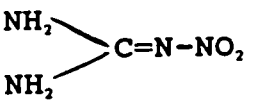
II. GENERAL INFORMATION

Nitroguanidine (NQ), a guanyl nitramine or nitramino compound, is a colorless, crystalline solid that may exist in two tautomeric forms. The alpha form, usually produced during manufacture of NQ, consists of long, thin, flat needles that are tough and difficult to pulverize; this form predominates in acidic, neutral, or slightly basic media. The beta form crystallizes from water in clusters of small, thin, elongated plates.

General chemical and physical properties of NQ are presented in Table II-1. NQ has approximately 77% the explosive power of trinitrotoluene (TNT), although at a density of 1.55, it has a higher rate of detonation (7,650 meters per second) than TNT has at the same density (6,900 meters per second). Nitroguanidine begins to undergo decomposition at a higher temperature (232°C) than does TNT (180 to 200°C); both are essentially nonhydroscopic, on the same order of stability, and soluble in water (Department of the Army and Air Force, 1967). On reduction, nitroguanidine yields nitrosoguanidine, which has been shown to be a gastric carcinogen in rats. NQ imparts a bitter, biting taste to water at a threshold concentration of 50 mg/L; this taste has been found to remain for 20 days at 20°C (Korolev et al., 1980).

NQ was used in military munitions during World Wars I and II. Because it has a low temperature of explosion, NQ is presently used in U.S. Army triple-base propellant mixtures. Its presence in the mixtures provides thrust and stability while reducing the burning temperature and flash intensity (Kenyon, 1982). It has been reported that when 10 to 15% NQ is incorporated into the propellant composition, the resulting propellant is almost flashless, and gases produced by the explosion are less erosive than those produced by other propellants of comparable force. When used in antiaircraft guns, this propellant increased the barrel life of a gun from 1,700 firings to 15,000 firings (Encyclopedia of Explosives and Related Items, 1974).

Table II-1. General Chemical and Physical Properties of Nitroguanidine[a]

Property	Value
CAS No.	556-88-7
Synonyms	Alpha-nitroguanidine Beta-nitroguanidine Guanidine-1-nitro Guanidine-nitro NG NGu NQ[b] Nitroguanidine N"-nitroguanidine N(1)-nitroguanidine Picrite
Molecular weight	104.074
Empirical formula	$CH_4N_4O_2$
Structure	
Physical state	Colorless, crystalline solid; exists in two forms: (1) alpha--long, thin, flexible, lustrous needles; and (2) beta--small, thin, elongated plates
Specific gravity	1.81
Melting point	Decomposes between 232°C and 245°C
Heat of combustion	209 kcal/mole
Density	1.72 g/cm^3
Vapor pressure	1.43×10^{-11} (25°C)
Stability characteristics	Alpha is stable form; sensitive to ultraviolet light (maximum absorption at 264 nm); explosive when shocked or exposed to heat or flame

(continued)

Table II-1. (cont.)

Property	Value
Log octanol-water partition coefficient (k_{ow})	-0.83
Solubility characteristics (alpha form):	
Water	4.4 g/L at 25°C 82.5 g/L at 100°C
Base (in potassium hydroxide)	12 g/L at 25°C
Acid (40% H_2SO_4)	80 g/L at 25°C
Alcohol	Slightly soluble
Ether	Insoluble

[a]SOURCE: Adapted from Burrows et al. (1989); Kenyon (1982); Department of the Army and Air Force (1967).
[b]NQ is used sometimes in lieu of NG to prevent confusing the latter abbreviation with another explosive, i.e., nitroglycerol.

Nitroguanidine is currently manufactured at the Sunflower Army Ammunition Plant in DeSoto, KS, by the initial combination of calcium cyanamide and ammonium nitrate. This mixture produces the intermediate material, guanidine nitrate, which is converted to NQ by dehydration with sulfuric acid (Fields and Rosenberg, 1984). By-products of this process include calcium nitrate, ammonia, and weak sulfuric acid. Information on production volume is not available.

III. SOURCES OF EXPOSURE

Occupational exposure to NQ may occur during manufacture and munitions incorporation. In addition, since NQ is soluble in water to 4.4 mg/mL, quantities that enter the environment via discharge streams from handling facilities present the potential for environmental contamination (Kaplan et al., 1982). The wastewaters could contaminate groundwater, soil for agricultural purposes, and public drinking water supplies. However, quantitative data are not available.

Wastewaters resulting from manufacture and loading of NQ may be discharged into the environment and present a potential for aquatic pollution. Seepage into the groundwater may occur from sediment deposits generated in Army ammunition plants. No information is available on the release of NQ during munitions use.

Direct contact with NQ may cause burns to skin and eyes. An NQ-fueled fire may produce poisonous gases that are irritating to the mucous membranes; runoff from fire control may also present a potential for aquatic pollution (DOT, 1984).

In concentrations greater than 2.5 mg/L, NQ has been found to retard the mineralization of organic water pollutants because of its bacteriostatic effect on saprophyte microflora (Korolev et al., 1980). This effect is characteristic of nitrogen-containing compounds.

IV. ENVIRONMENTAL FATE

Spanggord et al. (1985, 1987) conducted literature reviews, laboratory screening studies, and detailed investigations to identify dominant loss and movement processes for nitroguanidine and two other propellants produced or used by the U.S. Army. These studies identified photolysis and biotransformation as the dominant transformation routes for NQ. No products resulting from biotransformation of NQ were observed to build up in the medium. Cyanamide appeared to be an end product of NQ utilization.

A. SORPTION ON SOILS AND SEDIMENTS

Spanggord et al. (1985) calculated the sorption partition coefficient to be <0.1 for the sediments studied and the average octanol-water partition coefficient to be 0.148 ± 0.001. It is concluded that adsorption on soils and sediments is not a significant environmental fate of NQ.

B. HYDROLYSIS

The hydrolysis of NQ is extremely slow under most environmental conditions (Spanggord et al., 1985).

C. PHOTOLYSIS

Spanggord et al. (1985) investigated the sunlight photolysis of NQ in several natural waters and in pure water. The natural waters were Kansas River water, Searsville Lake water, and water containing commercial humic acids. The experiments were conducted in March. The light flux during each photolysis set was measured with a p-nitroacetophenone (PNAP) actinometer. Nitroguanidine photolyzed at very similar rates in all the waters, with a half-life of about 50 hours. The conditions of the study and the data obtained are summarized in Tables IV-1 and IV-2. Spanggord et al. (1985) also calculated the photolysis half-life of NQ in different seasons to be 3.9, 2.0, 1.0, and 1.6 days in winter, spring, summer, and fall, respectively.

Table IV-1. Sunlight Photolysis of NQ in Different Natural Waters[a]

Water[b]	pH	Absorbance (313)	$10^6 \times k_p$/sec[c]	Half-life (hr)
W	6.97	0.0016	4.31	45
KR	8.26	0.0311	3.39	57
SNW	8.64	0.0382	3.70	52
SL	8.33	0.0384	3.39	57

[a][NQ] = 3.86×10^{-6} M; absorbance (313) = 0.003 a.u.
[b]W = pure water; KR = Kansas River water; SNW = synthetic natural water; SL = Searsville Lake water.
[c]Photolysis rate constant (p-nitroacetophenone (k_p(PNAP)) = 3.08×10^{-6}/sec.

SOURCE: Spanggord et al. (1985).

Table IV-2. Sunlight Photolysis of High Concentrations of NQ in Different Natural Waters[a]

Water[b]	$10^6 \times k_p$/sec[c]	Half-life (hr)
W	3.86	50
KR	4.73	41
SNW	4.46	43
SL	4.06	47

[a][NQ] = 3.09×10^{-5} M; absorbance (313) = 0.022 a.u.
[b]W = pure water; KR = Kansas River water; SNW = synthetic natural water; SL = Searsville Lake water.
[c]Photolysis rate constant (p-nitroacetophenone) (k_p (PNAP)) = 3.33×10^{-6}/sec.

SOURCE: Spanggord et al. (1985).

Noss and Chyrek (1984) reported the photolysis products of NQ to be dependent on pH; guanidine and nitrate ion are products when the pH is between 3 and 10. In their Phase II study, Spanggord et al. (1987) conducted experiments to determine the photochemical products, the first-order photolysis rate constant (k_p), and the photochemical quantum yield of NQ. The quantum yield for NQ was calculated to be 0.011; k_p and half-life ($t_{1/2}$) were calculated for cloudless conditions and are summarized in Table IV-3. The k_p was calculated to be 1.0/day, and the $t_{1/2}$ was calculated to be 0.70 days. Half-lives of 2.8, 0.6, 1.3, and 2.3 days were calculated for NQ for the spring, summer, fall, and winter, respectively, at 40°N latitude. These values were obtained based on the light intensity for other seasons and on an average quantum yield of 0.011, and agree with the results obtained by Dennis (1982, as cited in Spanggord et al., 1985, 1987) as well as with the Phase I study by Spanggord et al. (1985). Both Spanggord studies concluded that the photolysis of NQ is not sensitized by naturally occurring humic substances. In fact, humic substances may decrease NQ photolysis rates because of a screening effect in which absorbing systems effectively compete for photons, thereby decreasing their availability to NQ. In their Phase II experiments, Spanggord et al. (1987) assumed that NQ would be present as an uncharged species at environmental pH as reported by Charton (1985). They predicted and identified nitrate and nitrite ions as transformation products produced during photolysis. No evidence was found for the formation of guanidine. It is concluded that the photochemical transformation initially yields nitrite and hydroxyguanidine. Nitrite is photochemically transformed to nitrate. Hydroxyguanidine undergoes sensitized photolysis and leads to unidentified ultimate products other than guanidine.

D. BIOTRANSFORMATION

Nitroguanidine is not susceptible to aerobic biodegradation in activated sludge but is reduced to nitrosoguanidine by anaerobic cultures (Kaplan et al., 1982). Ho et al. (1988) reported that it may be possible for NQ to undergo reduction <u>in vivo</u>. Using organisms collected from NG-holding ponds at Sunflower Army Ammunition Plant (SAAP),

Table IV-3. Nitroguanidine Environmental Photolysis Quantum Yield, Rate Constants, and Half-Lives[a]

Irradiation date (1986)	Solvent	S[b]	NQ Integral $e_L I_L$/day[e]	PNAP[g] Integral $e_L I_L$/day[e]	Quantum yield (PNAP)	Quantum yield (NQ)	k_p[h] (per day)	$t_{1/2}$ (days)
May 16-22	DW and KR water[c]	4.81±0.45	79.0	430	4.82×10^{-4}	0.0126	1.00±0.09	0.70
	HS[d]	3.13±0.32	--[c]	--	--	--	0.65±0.07	1.1
July 21-24	DW and KR water	1.91±0.09	100.4	532	1.0×10^{-3}	0.0102	1.02±0.05	0.68

[a]All experiments at pH 7.8, the natural pH of Kansas River water.
[b]Regression estimate slope of a plot of ln[NQ][NQ]$_o$ vs. ln[PNAP]/[PNAP]$_o$ ± 95% confidence limits of slope estimated, where [ ]$_o$ = initial concentration.
[c]DW = Distilled water with 5 mM phosphate buffer; KR = Kansas River water with absorbance at 313 nm = 0.39/cm.
[d]Aldrich humic solution, 60 mg/L; total organic carbon = 24 mg/L, absorbance at 313 nm = 0.66/cm.
[e]e_L = molar absorptivity at wavelength L; I_L = sunlight intensity at wavelength L.
[f]No results given.
[g]PNAP = p-nitroacetophenone.
[h]k_p = photolysis rate constant.

SOURCE: Spanggord et al. (1987).

Spanggord et al. (1985) determined that the biotransformation of NQ could occur under both aerobic and anaerobic conditions. The biotransformation was shown to be a cometabolic process in which the organisms could degrade NQ only in the presence of other organic nutrients (such as nutrient broth) and not as a sole carbon and energy source. The biotransformations were performed in shaker flasks, using nutrient broth as a cosubstrate in natural waters collected from SAAP. When the flasks were returned to static conditions, the reaction rate became rapid. Under static conditions, 90% of a 20-ppm NQ solution containing 200 ppm nutrient broth and 1 g/L phosphate buffer was transformed in 2 days. It appears that under static conditions, the microbes rapidly reduce the dissolved oxygen content, thereby creating a microaerophilic environment that appears to be the most favorable condition for the biotransformation of NQ. In their Phase II study, Spanggord et al. (1987) conducted a rate constant study using a high cell population of natural organisms acclimated to NQ in nutrient broth. Table IV-4 gives the results of NQ biotransformation first- and second-order rate constants as a function of NQ and nutrient concentration.

The average second-order rate constant (k_{b_2}) for microbes was 3.8 ($\pm$ 0.9) x 10^{-10} mL/organism/hour without additional nutrients; 1.5 ($\pm$ 0.4) x 10^{-9} mL/organism/hour with 20 ppm nutrients; and 2.3 ($\pm$ 0.3) x 10^{-9} mL/organism/hour with 50 ppm nutrients. From the second-order rate constants, it was possible to estimate NQ persistence under specific environmental conditions. In a quiescent water containing 1 x 10^6 organism/mL and a low nutrient level, a first-order rate constant was calculated to be 3.8 x 10^{-4}/hour, and the half-life was determined to be 85 days. In a pond bottom containing decomposed organic matter, the microbial population may be in the range of 1 x 10^7 organism/mL. Assuming 100 ppm organic matter, the first-order rate constant will be [2.3 x 10^{-9} mL/organism/hour) (1 x 10^7)] 2.3 x 10^{-2}/hour and the half-life will be 30 hours (Spanggord, 1987). Spanggord et al. (1987) could not detect nitrosoguanidine during their HPLC monitoring of NQ biotransformation. A GC/MS analysis identified cyanamide as one end product of NQ biotransformation. It is concluded, therefore, that

Table IV-4. NQ Biotransformation Rate Constants as a Function of NQ and Nutrient Concentration

[NQ] (ppm)	[NB]+[YE][a] (ppm each)	$k_{b_1}^{b}$ (hr^{-1})	[X][c] (organism/mL)	$k_{b_2}^{d}$ (mL/organism/hour)
2.1	0	0.033	8.40×10^{7}	4.0×10^{-10}
	20	0.102	8.40×10^{7}	1.2×10^{-9}
	50	0.192	8.40×10^{7}	2.3×10^{-9}
8.5	0	0.078	1.67×10^{8}	4.6×10^{-10}
	20	0.303	1.67×10^{8}	1.8×10^{-9}
	50	0.341	1.67×10^{8}	2.0×10^{-9}
11.5	0	0.028	1.01×10^{8}	2.8×10^{-10}
	50	0.265	1.01×10^{8}	2.6×10^{-9}

[a]NB = Nutrient broth, YE = Yeast extract.
[b]k_{b_1} = first order rate constant.
[c][X] = microbial concentration.
[d]k_{b_2} = second order biotransformation rate constant.

SOURCE: Spanggord et al. (1987).

cyanamide appears to be an end product of NQ utilization by microbes, and that there appears to be no buildup of detectable intermediate products. The microbial persistence of NQ is dependent on the environment with regard to the oxygen and nutrient concentrations. The measured rate constants are useful only as initial estimates of NQ persistence in aquatic environments.

V. PHARMACOKINETICS

A. ABSORPTION

Data suggest that NQ is rapidly absorbed by rodents following oral administration. Tissue distribution and pharmacokinetics studies were conducted by Morgan et al. (1985) and reported by Ho et al. (1988). Groups of 12 rats (6 males, 6 females) were used for the tissue distribution study; groups of 3 males were used for the pharmacokinetics study. Urine was measured at 4, 8, 24, 32, and 48 hours; feces were measured at 24 and 48 hours following dosing. Ho et al. (1988) reported that 40 to 50% of the radiolabel was excreted in the urine within 4 hours after dosing, 62 to 81% within 8 hours, 90 to 102% within 24 hours, and 93 to 104% within 48 hours (Ho et al., 1988; Morgan et al., 1985a); [^{14}C]NQ was not recovered in expired air. The route of administration, intravenous (iv) or oral, did not appear to influence the absorption of the radiolabel.

The blood half-life ($t_{\frac{1}{2}}$) for the elimination phase in all dose groups was between 1.8 and 3.2 hours. The blood concentration of NQ in all orally dosed rats peaked within 1.5 hours. The volumes of distribution were equivalent for all dose groups. The oral bioavailability of NQ was 100%. The absorption of [^{14}C]NQ in males and females dosed with 20 or 200 mg/kg [^{14}C]NQ was equal, indicating no dose dependency. No significant differences in NQ disposition were found between males and females or between the subchronically treated group and the single oral dose group.

B. DISTRIBUTION

Limited information was found regarding the distribution of NQ following intravenous or oral administration of the chemical. Ho et al. (1988) reported that 1 hour after an oral dose of 20 mg NQ/kg, radioactivity was concentrated primarily in the gastrointestinal (GI) tract (114 μg/g) but was distributed in the blood to the major organs, i.e., liver (22 μg/g), kidneys (28 μg/g), spleen (18 μg/g),

heart (18 μg/g), and lungs (17 μg/g) (Table V-1). The whole-blood radioactivity level for male and female Sprague-Dawley rats was 21.4 μg/g within 1 hour; this peaked within 1.5 hours. NQ distribution to the brain was not significant (5.9 μg/g). Within 48 hours after dosing, no significant radioactivity (<0.02% of the administered dose) remained in any major organ.

C. EXCRETION

Elimination of radioactivity was rapid following administration of a single oral dose of 20 or 200 mg [^{14}C]NQ/kg to male and female Sprague-Dawley rats or a single iv dose of 20 mg [^{14}C]NQ/kg (Ho et al., 1988; Morgan et al., 1985). By 4 hours after administration of the test material, rats had excreted 40 to 50% of the administered radiolabel in the urine; between 93.4 and 103.9% of the administered radiolabel was recovered in the urine of all dosed animals within 48 hours (Table V-2). The small amount of radiolabel recovered in the feces (0.4 to 1.6%) was considered to be due to urine contamination. Labeled carbon dioxide [^{14}C]CO_2 was not detected in expired air following administration of [^{14}C]NQ. Morgan et al. (1985) suggested that NQ possesses characteristics of an osmotic diuretic.

D. METABOLISM

No metabolism of NQ was found <u>in vivo</u> (Morgan et al., 1985). Data indicated that NQ was eliminated by urinary excretion rather than detoxified by hepatic metabolism. Following oral administration of [^{14}C]NQ to rats, approximately 100% of the administered radioactivity was found in the urine within 48 hours as unchanged NQ (Morgan et al., 1985; Ho et al., 1988).

When N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was orally administered to albino rats at doses of 100 mg/kg in dimethylsulfoxide (DMSO), NQ was reported as a secondary urinary metabolite (Tanaka and Sano, 1971). It was hypothesized that MNNG reacted with an amino group to produce NQ <u>in vivo</u>. The methylating potential of MNNG is not found in NQ.

Table V-1. Tissue Levels of Radioactivity 1 Hour Following Oral Administration of 20 mg [^{14}C]NQ/kg to Rats[a]

Tissue	NQ radioactivity	
	Tissue (μg/g)	Tissue:blood ratio
Liver	22.1	1.03
Kidney	28.0	1.31
GI tract (and contents)	114.0	5.33
Spleen	18.4	0.86
Heart	17.8	0.83
Brain	5.9	0.28
Lung	17.2	0.80
Skeletal muscle	16.3	0.76
Whole blood	21.4	NA[b]

[a]Based on three rats with the exception of GI tract data, which are based on two rats.
[b]Not applicable.

SOURCE: Ho et al. (1988).

Table V-2. Distribution and Excretion of Radioactivity 48 Hours After Administration of [^{14}C]NQ to Rats[a]

Endpoint	Percentage of administered radioactivity (mean + S.D.)		
	Group A (iv, 20 mg/kg)	Group B (oral, 20 mg/kg)	Group C (oral, 200 mg/kg)
		Males	
Major organs[b]	0[c]	0	0
Whole blood	0	0	0
Expired air[d]	—[e]	—	—
Feces[f]	0.7 ± 0.4	1.6 ± 1.3	0.7 ± 0.2
Urine	93.4 ± 4.3	99.5 ± 6.9	98.3 ± 2.8
Total recovery	94.1	101.1	99.0
		Females	
Major organs[b]	0	0	0
Whole Blood	0	0	0
Expired air[d]	—	—	—
Feces[f]	0.6 ± 0.4	1.2 ± 0.7	0.4 ± 0.1
Urine	96.1 ± 4.2	103.9 ± 3.2	97.8 ± 6.1
Total recovery	96.7	105.1	98.2

[a]Based on six rats/sex/dose.
[b]Organs analyzed include liver, lung, kidney, spleen, brain, skeletal muscle, testes, and ovaries.
[c]Zero values indicate levels <0.02% of administered radioactivity.
[d]No radiolabel was found in expired CO_2 of pilot study animals; subsequent studies did not include expired air measurements.
[e]Not available.
[f]Urine contaminated.

SOURCE: Ho et al. (1988).

VI. HEALTH EFFECTS

A. HUMANS

No studies on the health effects of NQ in humans have been found in the literature.

B. ANIMAL EXPERIMENTS

1. Short-term Exposure

Few short-term laboratory studies on NQ were found in the available literature. For this reason, the toxicities of the following pertinent associated compounds have also been reviewed and are presented in Appendix C: (1) guanidine hydrochloride (GuHCL), the hydrochloride salt of guanidine and a by-product of NQ; and (2) guanidine nitrate (GuN), an intermediate and by-product of NQ. A sample of effluent wastewater from an NQ processing plant was evaluated by American Cyanamid (1955), and the results are presented.

a. Acute

1) Nitroguanidine

Acute LD_{50} values for nitroguanidine are summarized in Table VI-1. The acute oral LD_{50} value for NQ was found to be greater than 4.64 g/kg when administered by gavage to groups of five male albino rats at 0.46, 1.00, 2.15, or 4.64 g/kg (American Cyanamid, 1955). NQ was nontoxic at low doses; however, one death was reported at the high dose. Clinical signs of depression, bloody nasal discharge, dyspnea, and diarrhea were noted in the four surviving high-dose males. In addition, two of the four high-dose males exhibited ataxia and one exhibited tremors. All animals appeared normal within 72 hours after dosing. No significant gross pathology was reported in dosed animals with the exception of the single high-dose death. Gastrointestinal irritation, hemorrhage of the intestine and urinary bladder, and congestion of the kidneys and adrenal were found at necropsy of this animal. Body weight data were not reported.

Table VI-1. Acute LD_{50} Values for Nitroguanidine in Laboratory Animals

Test Substance	Species	Strain	Sex	Route	Vehicle	LD_{50} (g/kg)	Reference
NQ	Rat		M	oral	Water	4.64	American Cyanamid (1955)
NQ	Mouse Rat Guinea pig			oral	Sunflower seed oil	3.85 10.20 3.12	Korolev et al. (1980)
NQ	Mouse	ICR	M F	oral	Methylcellulose/Tween-80	>5.62 (MLD)[a] 4.34 (MLD)	Hiatt et al. (1988a,b)
	Rat	Sprague-Dawley	M F	oral oral	Methylcellulose/Tween-80	>5.62 (MLD) >5.62 (MLD)	Hiatt et al. (1988a,b)
NQ effluent	Rat		M		--[b]	>21.5 mL/kg	American Cyanamid (1955)

[a]MLD = Median lethal dose.
[b]No vehicle.

Korolev et al. (1980) studied the acute toxicity of NQ in mice, rats, and guinea pigs administered NQ perorally in solutions of sunflower seed oil. The acute oral LD_{50} of NQ was found to be 3.85, 10.20, and 3.12 g/kg for white mice, white rats, and guinea pigs, respectively. Clinical patterns of intoxication were similar to those resulting from exposure to diphenylnitrosamine and were characterized by the development of cyanosis. NQ was classified to be of moderately low toxicity. No further information was reported.

The median lethal dose (MLD) for NQ was found to be 4.34 g/kg for females and greater than 5.62 g/kg for males when administered by oral intubation to groups of 10 ICR mice at doses of 5.62 g/kg (males) or 6.31, 5.01, 3.98, and 2.82 g/kg (females) (Hiatt et al., 1988a,b). NQ was prepared as a suspension in 0.2% methylcellulose and 0.4% Tween-80. Four males receiving 5.62 g/kg and nine, six, three, and two females receiving 6.31, 5.01, 3.98, and 2.82 g/kg, respectively, died during the study. Of these, 2 (50%) of the males and 11 (55%) of the females died within 24 hours of dosing (Table VI-2). Clinical signs of toxicity produced by NQ included signs associated with effects on the central nervous system (CNS). Two types of seizures were exhibited in females in a dose-related manner; handling-induced seizures were tonic/extensor, and spontaneous seizures were clonic. Females appeared to be more susceptible to seizure induction than males (Table VI-3). No seizures were exhibited in males receiving 5.62 g/kg, but females receiving 5.01 and 3.98 g/kg (doses that bracketed an equal degree of mortality) exhibited a 40% incidence of seizures. Hyperactivity and exaggerated startle reflex behavior were also exhibited in females at lower incidences. However, no abnormal histopathology was found in these animals at necropsy. Hunched posture and inactivity were the most common clinical signs in males and females and were considered by the study authors to reflect effects on the gastrointestinal tract (Table VI-3).

No gastrointestinal effects were found histologically in males or females. Of 10 dosed males, 3 exhibited perianal staining; a white crystalline precipitate, presumed to be NQ, was found in the urine of 4/10 males and 22/40 females. No evidence of impaired renal function was

Table VI-2. Mortality of ICR Mice Dosed Orally With Nitroguanidine[a,b]

Dose level (g/kg)	Number of compound-related deaths	% Mortality
	Males	
0	0	0
5.62	4	40
	Females	
0	0	0
2.82	2	20
3.98	3	30
5.01	6	60
6.31	9	90

[a]To keep volume of dosing solutions below 10 mL/kg, all test animals received split dosings, by gavage, within 90 minutes' duration. Half of the vehicle control animals received split dosings; half received a single dose by gavage.
[b]Based on 10 mice/group; control females had 9 mice/group.

SOURCE: Hiatt et al. (1988a).

Table VI-3. Incidence of Clinical Observations in Mice Dosed Orally With Nitroguanidine[a]

Clinical signs	Dose level (g/kg)						
	Males		Females				
	0	5.62	0	2.82	3.98	5.01	6.31
Hunched posture	0	8	0	6	7	5	9
Inactivity	0	7	0	4	5	6	8
Rough coat	0	3	0	0	0	0	0
Seizures	0	0	0	2	4	4	9
White crystalline material in urine	0	4	0	9	3	3	7
Yellow perianal staining	0	3	0	0	0	0	0
Irritability	0	0	0	1	3	0	1
Hyperactivity	0	0	0	0	3	0	0

[a]Based on 10 mice/group; control females had 9 mice/group.

SOURCE: Hiatt et al. (1988a).

reported histologically for animals that died during the study. Body weights were similar in dosed and control males and females. NQ was classified to be "practically nontoxic" to "slightly toxic" because a dose exceeding the maximum tolerated dose produced less than 50% mortality in male mice, and the MLD in females (4.34 g/kg) was close to the maximum tolerated dose of 5.00 g/kg.

Hiatt et al. (1988b) reported that when 5.0 g NQ/kg was administered by oral gavage to Sprague-Dawley rats, 2/7 males (29%) and 2/6 females (33%) died during the study. Of 13 rats, 6 were reported to exhibit clinical signs of gastric and intestinal irritation that were confirmed at necropsy. A white, crystalline material, identified as NQ, was found in the urine and feces of all dosed animals. No CNS effects were observed. The study authors reported that a significant fraction of the test material was excreted unchanged in the urine. No urinary tract or renal effects were found histologically.

Hiatt et al. (1988b) and Lewis et al. (1988) studied the acute dermal toxicity of NQ in New Zealand White rabbits. Doses of 2.0 g NQ/kg (in a slurry concentration of 0.25 g NQ/mL saline vehicle) applied to the dorsal and lateral intact skin of five male and five female rabbits resulted in no deaths and produced no compound-related systemic toxicity or dermal irritation. Erythema observed during the study was occasional and was considered to be the result of parasitic infections. NQ was reported to possess a minimum potential for acute dermal toxicity.

No signs of systemic toxicity or primary skin irritation were found when albino rabbits received dermal applications of NQ paste at doses of 1.00, 2.15, 4.46, or 10 g/kg for 24 hours (American Cyanamid, 1955). No significant gross pathology was reported.

2) Nitroguanidine effluent

A sample of effluent wastewater from an NQ processing plant containing 1.96% total solids by weight was administered to albino rats and rabbits for acute oral, dermal, and subchronic feeding studies.

The analyzed sample (pH 6.3, biochemical oxygen demand 7,700 ppm) was also found to contain 0.51% ammonia nitrogen, 0.013% nitrate nitrogen, 0.88% sulfate, 0.019% chloride, and a trace of phenol. Dissolved components from neutralized waste effluent from NQ manufacture included ammonium sulfate, calcium nitrate, guanidine sulfate, NQ, melamine, thiourea, cyanamide, dicyandiamide, urea, and sulfide as H_2S. Undissolved components from this same sample included calcium sulfate, carbon, calcium carbonate, aluminum, iron and silicon oxides, and calcium fluoride (American Cyanamid, 1955).

The undiluted effluent was toxic only at high concentration with an LD_{50} greater than 21.5 mL/kg when administered, by gavage, to groups of five male albino rats at 1.00, 2.15, 4.64, 10.0, or 21.5 mL/kg (American Cyanamid, 1955). No signs of systemic toxicity were reported.

No symptoms of systemic toxicity or primary skin irritation were observed when groups of four albino rabbits received single dermal applications of undiluted effluent at doses of 1.00, 2.15, 4.64, or 10.00 mL/kg for 24 hours (American Cyanamid, 1955).

b. Primary irritation and dermal sensitization

Using the modified Draize method for skin irritation, an NQ paste (0.5 g NQ and sufficient 0.9% physiological saline to produce a thick paste) was applied directly to the shaved, intact skin of four male and four female New Zealand White rabbits and evaluated at 1, 24, 48, and 72 hours (Morgan et al., 1986b). Nitroguanidine was classified as a nonirritating chemical, based on negative findings from this study. It was also reported that the compound was minimally absorbed, and most of the NQ remained on the skin following patch removal.

A dosage of 0.5 g NQ (moistened with saline) was applied to the shaved intact dorsal surface of New Zealand White rabbits for 4 hours (Hiatt et al., 1988b). No signs of dermal irritation, erythema, or edema were observed during the 14-day observation period.

Using the modified Draize method for eye irritation, 0.025 g of crystalline, powdered NQ was applied directly to the eye of three male and three female New Zealand White rabbits and evaluated at 1, 4, 24, 48, and 72 hours (Hiatt et al., 1986). The contralateral eyes of each rabbit served as the untreated controls. Slight conjunctival inflammation was reported in five of the six rabbits (83%) at 1 and 4 hours posttreatment. Undissolved NQ was found in the eyes of these rabbits, indicating that physical irritation may have been responsible for the observed effect. One rabbit with diffuse stippling of the cornea prior to dosing exhibited a corneal punctate lesion 48 hours following treatment. These reactions were considered to be minor; NQ was not found to be an eye irritant.

Hiatt et al. (1988b) instilled 0.25 g NQ into the eye of six New Zealand White rabbits; evaluations were performed at 1, 4, 24, 48, and 72 hours. Slit lamp examinations were performed at 24, 48, and 72 hours with special attention given to the corneal surface and thickness, clarity of the anterior chamber fluid, and iridal and lens surface morphology. No significant signs of eye irritation were exhibited; 5/6 rabbits developed slight conjunctival vasodilation, indicative of mild inflammation, at 1 and 4 hours following dosing. This response did not qualify as a positive reaction and no other signs of ocular irritation were found.

Using the Buchler dermal sensitization method, a 10% solution of NQ in 0.9% saline was applied to the shaved skin of 10 male Hartley guinea pigs for 6 hours, once each week for 3 consecutive weeks during the induction phase (Hiatt et al., 1988b). Two weeks following the third induction dose, a challenge dose of the test compound was applied to the induction site; the site was left uncovered to negate local effects produced from repeated exposure. A negative control group of 10 animals receiving only the challenge dose was included to detect any response due to direct dermal irritation. A positive control group was treated with dinitrochlorobenzene (DNCB) in the same manner as the group treated with the test compound. Exposure to NQ showed no evidence that indicated dermal sensitization.

c. Subacute

1) Nitroguanidine

The toxic effects of subacute (14-day) dietary administration of NQ were studied in Sprague-Dawley rats (Morgan et al., 1988a). NQ was administered in the daily diet of 10 rats/sex/dose for 14 days at 0, 100, 316, and 1,000 mg/kg/day; no deaths occurred during the study. Body weights of high-dose females were significantly ($p < 0.05$) decreased when compared with concurrent controls at the end of the acclimation period prior to study initiation; this weight decrease, which persisted throughout the study, was due to inadvertent water deprivation and was not considered to be compound related. Water consumption increased in a dose-related manner in males and females throughout the study; this increase was significant in males and females fed 316 and 1,000 mg/kg/day. Food consumption was not affected. Serum potassium levels were found to be significantly ($p < 0.05$) decreased in high-dose males; this decrease was slight in high-dose females (Table VI-4). Serum calcium levels were decreased in all dosed males; this decrease was significant ($p < 0.05$) at the low dose. Absolute heart weights and heart-to-brain weight ratios exhibited a dose-related decrease and were found to be significantly ($p < 0.05$) decreased in high-dose females; no organ weight changes were exhibited in males (Table VI-5). Gross and microscopic histopathology revealed no compound-related lesions. Morgan et al. (1988a) considered the findings of increased water consumption and decreased serum electrolytes to indicate that NQ, which is excreted unchanged in the urine of the rat, may be acting as an osmotic diuretic. Based on increased water consumption and decreased electrolytes in high-dose males and females and reduced heart weights in high-dose females, the LOAEL is 1,000 mg/kg/day and the NOAEL is 316 mg/kg/day.

No significant effects on survival, food intake, weight gain, or gross pathology were noted when groups of 10 male albino rats (strain not specified) were fed 0.01, 0.10, or 1.0% NQ, equivalent to mean daily dosages of 0.01, 0.1, or 0.93 g/kg, respectively, for 30 days (American Cyanamid, 1955). One high-dose male died after 20 days of feeding, and one mid-dose male died after 27 days of feeding; however, these deaths were

Table VI-4. Selected Serum Electrolyte (mean ± SE) Levels in Rats Administered NQ for 14 Days

Parameter	Dose level (mg/kg/day) 0	100	316	1,000
		Males		
Sodium (mEq/dL)	182.1 ±5.7	182.1 ±3.4	178.1 ±4.0	169.0 ±5.5
Potassium (mEq/dL)	8.02 ±0.52	7.48 ±0.21	7.71 ±0.28	6.57* ±0.27
Chloride (mEq/dL)	112.7 ±1.3	109.7 ±1.4	110.2 ±2.0	113.8 ±1.8
Calcium (mg/dL)	11.99 ±0.21	11.23* ±0.08	11.61 ±0.20	11.51 ±0.19
Phosphorus (mg/dL)	14.3 ±0.4	13.4 ±0.3	13.6 ±0.4	13.1 ±0.4
Magnesium (mg/dL)	3.27 ±0.16	3.08 ±0.15	2.94 ±0.09	2.96 ±0.10
		Females		
Sodium (mEq/dL)	165.6 ±2.4	166.7 ±1.9	166.9 ±1.1	163.1 ±2.4
Potassium (mEq/dL)	6.74 ±0.21	6.71 ±0.22	7.21 ±0.35	6.14 ±0.15
Chloride (mEq/dL)	122.2 ±2.5	122.5 ±1.6	122.6 ±1.4	118.8 ±0.9
Calcium (mg/dL)	10.78 ±0.16	10.96 ±0.12	10.92 ±0.19	10.58 ±0.11
Phosphorus (mg/dL)	10.4 ±0.5	10.7 ±0.4	10.6 ±0.3	9.4 ±0.4
Magnesium (mg/dL)	3.17 ±0.12	3.32 ±0.09	3.41 ±0.10	2.96 ±0.10

*Significantly different from control value ($p < 0.05$) by Dunnett's test.

SOURCE: Morgan et al. (1988a).

Table VI-5. Absolute and Relative Heart Weights (± SE) in Rats Administered NQ for 14 Days

Dietary level (mg/kg/day)	Mean heart weights		
	Absolute (g)	Relative[a] (%)	Relative[b] (%)
		Males	
0	1.12 ± 0.04	0.35 ± 0.01	57.7 ± 2.2
100	1.19 ± 0.04	0.37 ± 0.01	63.1 ± 3.0
316	1.29 ± 0.04*	0.39 ± 0.02	68.6 ± 1.9*
1,000	1.11 ± 0.04	0.34 ± 0.01	59.2 ± 1.4
		Females	
0	0.91 ± 0.03	0.37 ± 0.01	51.7 ± 2.0
100	0.87 ± 0.02	0.37 ± 0.01	50.0 ± 1.1
316	0.85 ± 0.04	0.36 ± 0.01	45.5 ± 1.6*
1,000	0.80 ± 0.03*	0.36 ± 0.01	43.8 ± 1.9*

[a]Percent of body weight.
[b]Percent of brain weight.
*Significantly different from control value ($p < 0.05$) by Dunnett's test.

SOURCE: Morgan et al. (1988a).

reported to be the result of a respiratory infection and were not considered to be compound related.

Korolev et al. (1980) conducted a 40-day subchronic study in which white rats (strain not reported) were dosed with 20, 100, or 500 mg NQ/kg. Body weight, organ weight, hematologic indices, limited clinical biochemistry including cholinesterase activity, and reproductive toxicity were studied. Results indicated that NQ effects are highly cumulative; peripheral blood, the central nervous system, and blood and liver enzymes were reported to be principally affected by the compound. No further information was reported.

2) Nitroguanidine effluent

No significant effects on survival, food intake, weight gain, or gross pathology were observed when 1.0, 2.5, or 5.0% undiluted effluent was fed to groups of 10 male albino rats (strain not specified) over a period of 30 days (American Cyanamid, 1955). The mean daily dosage of effluent solids was calculated to be 20, 50, and 95 mg/kg for the groups dosed at 1.0, 2.5, and 5.0%, respectively.

2. Longer-term Exposure

a. 13-Week studies

Morgan et al. (1988b) conducted a 90-day subchronic oral toxicity study of NQ in male and female Sprague-Dawley rats in which 15 rats/sex were randomly assigned to each of 4 dose groups. Selected on the basis of the results of an acute toxicity study and a 14-day subacute study, the dietary doses were 0, 100, 316, and 1,000 mg/kg body weight/day for 90 days. All diet mixtures were within 6.4% of the target concentration. Animals were observed twice daily, and body weights were recorded weekly and on the day of sacrifice. All animals were subjected to complete necropsy and histopathology at the interim (45 days, 5 rats/sex/dose) or terminal (90 days, 10 rats/sex/dose) sacrifice, following exsanguination for hematology and clinical chemistry measurements.

No deaths occurred during the study. Food consumption was sporadically decreased in dosed males and females; these decreases were slight and inconsistent. Water consumption was increased in a dose-related fashion for both males and females during the 13 weeks of the study; this increased water consumption was significant ($p < 0.05$, $p < 0.01$) at 1,000 mg/kg/day. Morgan et al. (1988b) considered these findings to indicate that NQ may have acted as an osmotic diuretic; the dose-related increases in water consumption were consistent with an increased urinary volume requirement for excretion of NQ. Females fed 1,000 mg/kg/day exhibited a decrease in mean body weight during weeks 5 through 13; this decrease was significant ($p < 0.05$, $p < 0.01$) (90% of control weight) at weeks 5, 6, 8, 9, and 12 (Table VI-6). No clinical signs that could be attributed to compound administration were observed. The brain-to-body weight ratio of high-dose females was significantly ($p < 0.05$) increased at 13 weeks, which correlated with the decrease in body weights of these animals. Absolute brain weights were not affected. A nonsignificant reduction in absolute and relative (organ to body weight, organ to brain weight) heart weights was exhibited in males in a dose-related manner. Heart weights of dosed females were similar to those of concurrent controls.

Clinical chemistry data revealed a significant decrease in serum lactic dehydrogenase (LDH) activity at terminal sacrifice in males fed 316 mg/kg/day and a significant increase in cholesterol at interim and terminal sacrifice in males fed 1,000 mg/kg/day. Females fed 100 and 1,000 mg/kg/day exhibited a significant decrease in mean triglyceride levels at terminal sacrifice. However, all clinical chemistry values in dosed animals were within normal limits. NQ had no effect on the hematological measurements in males. Significant decreases were noted in platelet counts at the interim sacrifice in females fed 100 mg/kg/day and in mean erythrocyte counts at terminal sacrifice in females fed 316 and 1,000 mg/kg/day when compared with concurrent controls. However, all hematology values of dosed males and females were within normal limits. No compound-related gross or microscopic lesions were seen. Based on decreased body weights in high-dose females and increased water consumption in high-dose males and females, the LOAEL is 1,000 mg/kg/day,

Table VI-6. Representative Results of Mean Body Weights (± SE) of Rats Administered NQ for 90 Days

Dietary level (mg/kg/day)	Mean body weights (g) at weeks: 5	9	12
	Males		
0	417 ± 7	477 ± 10	515 ± 9
100	415 ± 6	459 ± 13	505 ± 10
316	415 ± 9	460 ± 16	486 ± 19
1,000	398 ± 6	459 ± 10	496 ± 12
	Females		
0	270 ± 5	296 ± 8	316 ± 8
100	260 ± 6	291 ± 9	312 ± 9
316	263 ± 4	281 ± 7	297 ± 8
1,000	248 ± 4**	265 ± 7*	284 ± 8*

*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

SOURCE: Morgan et al. (1986a).

and the NOAEL is 316 mg/kg/day. Female rats appear to be more sensitive to NQ than male rats.

Frost et al. (1988) conducted a 90-day subchronic oral toxicity study of NQ in male and female ICR mice in which 15 mice/sex were randomly assigned to each of 4 dose groups. On the basis of an acute toxicity study and a pilot study, the doses administered were 0, 100, 316, or 1,000 mg/kg body weight/day in the diet for 90 days. Animals were observed twice daily, and body weights were recorded weekly and on the day of sacrifice. All animals were subjected to complete necropsy and histopathology at the interim (45 days, 5 rats/sex/dose) or terminal (90 days, 10 rats/sex/dose) sacrifice following exsanguination for hematology and clinical chemistry measurements.

No mortalities occurred during the study. No significant effects on food consumption were observed; water consumption was significantly increased in high-dose females at weeks 2 and 3 and high-dose males at weeks 1 through 13. No significant effects on body weight were seen. In addition, no compound-related clinical signs of toxicity were observed. Clinical chemistry data did not reveal any alterations attributable to dosing with NQ. Sporadic changes in various clinical chemistry parameters were not consistent and were within the normal range established by the baseline control sacrifice. Hematological data did not reveal any significant compound-related changes. Heart weights, heart-to-body weight ratios, and heart-to-brain weight ratios were decreased in a dose-related fashion in all dosed males at terminal sacrifice. At interim sacrifice, this group had a significantly greater brain-to-body weight ratio; absolute brain weights were not affected. These heart and brain weight changes were not found in female mice. No compound-related gross or microscopic lesions were observed at any dose. NQ may have been acting as an osmotic diuretic; the dose-related increases in water consumption were consistent with an increased urinary volume requirement for excretion of NQ. Based on reduced heart weights in high-dose males and increased water consumption in high-dose males and females, the LOAEL is 1,000 mg/kg/day and the NOAEL is 316 mg/kg/day.

b. Lifetime studies

Korolev et al. (1980) conducted a chronic toxicity study in which animals were dosed with 0.005, 0.05, or 0.5 mg NQ/kg. Animal species, method of administration, and duration of study were not reported. Dose levels were based on results of acute and subchronic studies, and they incorporated a correction factor for cumulative effects of the compound. In addition to the parameters tested for the 40-day subchronic study (described under section VI.B.1.c, Subacute), liver histamine, blood serum beta-lipoproteins, sulfhydryl groups, mutagenicity, and reproductive toxicity were studied. At the mid- and high-dose levels, NQ was found to cause significant ($p < 0.05$) changes in the "enzyme generating function" of the liver, hematological parameters, and the number of sulfhydryl groups in the blood. However, the nature of the changes was not specified. No genotoxic or reproductive effects were found at any dose tested. The NOEL was reported to be 0.005 mg/kg, the lowest dose tested. The maximum permissible concentration of NQ in bodies of water was reported to be 0.1 mg/L based on the lack of mutagenic or reproductive effects at 0.005, 0.05, or 0.5 mg NQ/kg. No further information was reported.

3. Reproductive Effects

No reproductive (gonadotoxic and mutagenic) effects were found after dosing with 20, 100, or 500 mg NQ/kg for 40 days or 0.005, 0.05, or 0.5 mg NQ/kg during a chronic toxicity study (Korolev et al., 1980). No further information was reported.

4. Developmental Toxicity

Coppes et al. (1988a) administered NQ in 1% carboxymethylcellulose (CMC) to sperm-positive (day 0) female rats. The animals were dosed on gestation days 6 through 15. The dosages given were 0, 100, 316, or 1000 mg/kg/day to groups of 27, 27, 23, and 27 animals, respectively. Dosing was based on the day 6 body weight. Eight females died during the dosing period; five of these deaths (two were nonpregnant females) were associated with gavage trauma in the groups dosed at 100 (one death), 316 (one death), and 1,000 (three deaths) mg/kg. The remaining three deaths

in the 1,000-mg/kg group were compound related. Signs of compound-related maternal toxicity were exhibited only in the 1,000-mg/kg group and included hunched posture, tremors, "irritability", "jitteriness", dehydration, and red urine. A statistically significant ($p < 0.05$) reduction in food consumption was observed in the high-dose dams during the dosing period, and significant decreases were observed in mean body weight and body weight gain (corrected for gravid uterine weight) through gestation for these same animals. At day 20 of gestation, 18 to 24 litters per dose group were collected by cesarean section. Embryotoxicity was evidenced by a nonsignificant increase in the resorption rate in the 1,000-mg/kg group; the mean resorptions/litter were 1.1, 1.0, 0.9, and 1.7 in the groups dosed at 0, 100, 316, and 1,000 mg/kg, respectively. The male and female fetuses in the 1,000-mg/kg group were significantly shorter and weighed significantly less than the control fetuses; the sex ratio was not affected. These animals exhibited an increased incidence of skeletal variations (retarded ossification of the sternebrae, caudal vertebrae, and pubis). Coppes et al. attributed these observations of retarded development to maternal toxicity rather than to the direct effect of NQ on the fetuses. Skeletal and soft tissue examination of the 316-mg/kg group showed cleft palate in one fetus each in two litters and a displaced eye with a reduced lens in one fetus of a third litter; each of these affected fetuses also showed one or more additional abnormalities (small jaw, hypoplastic lungs, bilateral anophthalmia, heart abnormalities); maternal toxicity was not observed at this dose level. Since the incidence of these malformations was low, Coppes et al. considered them to be incidental and unrelated to dosing. No malformations occurred in the 100- or 1,000-mg/kg groups. No evidence of developmental toxicity of NQ in rats was found under conditions of this study. The LOAEL is 1,000 mg/kg based on the maternal and fetal toxicity at the high dose, and the NOAEL is 316 mg/kg.

In the study with New Zealand White rabbits, Coppes et al. (1988b) administered NQ in 1% CMC by oral gavage to 17, 18, 16, and 22 mated (day 0) females at dosages of 0, 100, 316, or 1,000 mg/kg/day (5 mL/kg), respectively, on each of gestation days 6 through 18. Dosing was based on the day 6 body weight. All 10 deaths occurring during the study were mated females in the 1,000-mg/kg/day group that died on gestation days 9

through 19; signs of toxicity preceding death included hypertonia, ataxia, convulsions, hunched posture, and thick, foamy urine. Signs of compound-related maternal toxicity were not observed in the 100- or 316-mg/kg groups. Maternal food consumption was significantly ($p < 0.05$) reduced during the dosing period in the 1,000-mg/kg group, but consumption returned to control levels in the postdosing period. Maternal mean body weight change during the dosing period showed a dose-related decrease that was significant ($p < 0.05$) in the 1,000-mg/kg group; the mean changes were 0.26, 0.23, 0.18, and -0.10 kg in the groups dosed at 0, 100, 316, and 1,000 mg/kg, respectively. Compound-related effects were not seen in the day 29 mean body weights corrected for gravid uterine weights or in the corrected body weight change for days 0-29. Examination of 11-15 dams by cesarean section on gestation day 29 showed no effect on preimplantation losses or on the implantation rate. However, the number of resorptions/litter was significantly ($p < 0.05$) increased at all levels of NQ exposure but was considered to be of no biological significance. The percent resorptions/litter was 2.2, 9.3, 6.6, and 17.9 for females dosed at 0, 100, 316, and 1,000 mg/kg, respectively. The percent resorptions/litter for control rabbits was considered by the study sponsor to be lower than the percent found in historical control data. Since these historical data were not available at the study laboratory, a mean range of percent resorptions for control New Zealand White rabbits (3.3-16%) was obtained from the recent literature (see Appendix B). The percent resorptions/litter exhibited in dams treated with 100 and 316 mg NQ/kg/day was within this control range and was not considered to be biologically significant. Even though the percent resorptions/litter exhibited in dams treated with 1,000 mg NQ/kg/day was similar to the upper limits of this historical range, this finding was considered to be of biological and statistical significance (see also Appendix B). No significant differences in the number of dead fetuses/litter were noted among control and treated groups; dead fetuses were observed in 0/13, 3/15, 1/15, and 2/11 litters from dams dosed at 0, 100, 316, and 1,000 mg/kg, respectively. Fetal mean body weights of male and female offspring were significantly ($p < 0.05$) decreased in the 1,000-mg/kg group; in addition, the female fetuses in this dose group were nonsignificantly shorter in mean length (9.9 ± 0.6 cm) than the controls (10.5 ± 0.4 cm). No teratogenic effects were observed. Skeletal variations (delayed

ossification) showed a compound-related increase in the number of affected fetuses in the dosed groups; the number of fetuses affected/number examined was 46/121 (38%), 64/135 (47%), 61/131 (47%), and 52/87 (60%) in the groups dosed at 0, 100, 316, and 1,000 mg/kg, respectively. The increase was significant ($p < 0.05$) at 1,000 mg/kg/day. Based on maternal and embryotoxicity at 1,000 mg/kg/day, the LOAEL is 1,000 and the NOAEL is 316 mg/kg/day. The resorption data provide equivocal evidence that NQ is a developmental toxicant in rabbits. (The value of the percent resorptions in high-dose rabbits was incorrectly reported by the study authors to be 9.7; this value was corrected to 17.9.)

5. Carcinogenicity

No studies on the carcinogenicity of NQ were found in the literature.

6. Genotoxicity

The mutagenic potential of NQ was evaluated in studies using the Ames *Salmonella*/mammalian microsome mutagenicity assay. *Salmonella typhimurium* strains TA98, TA100, TA1537, and TA1538 were exposed to NQ, in the presence and absence of metabolic activation, at a concentration of 10 mg/L in one study (Ishidate and Odashima, 1977) and at levels of 5 to 5,000 μg/plate in a second study (Kaplan, 1982). Nitroguanidine was assayed up to an acceptable high dose in both studies with no indication of a mutagenic effect. McGregor et al. (1980) also reported NQ to be nonmutagenic when tested under the same conditions using the same test system; test concentrations were, however, not reported.

In the preincubation modification to the Ames assay conducted by Sebastian and Korte (1988), *S. typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were exposed to six nonactivated and six S9-activated concentrations of NQ ranging from 0.0875 to 2.8 mg/plate. NQ was neither cytotoxic nor mutagenic. The authors reported that the high dose (2.8 mg/plate) approached the limit of solubility of NQ in the preincubation mixture. In the absence of a cytotoxic effect, it is considered an acceptable practice to assay test materials up to a level that approaches the limit solubility. The results of this study are,

therefore, considered valid evidence of a negative response in this test system.

Harbell and Korte (1987) found that doses of NQ ranging from 0.01 to 4 mg/mL in an initial assay and doses ranging from 1 to 4 mg/mL in a confirmation assay were not cytotoxic, and that they did not increase the frequency of forward mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. Under both nonactivated and S9-activated conditions, the highest assayed dose was reported to approach the solubility limits for NQ in aqueous medium. The reviewers concluded that the study was well controlled, and the test material was assayed to an appropriate high dose with no indication of a mutagenic response.

NQ was also investigated for the potential to induce sex-linked recessive lethal mutations in Drosophila melanogaster (Gupta et al., 1988). In this study, Canton-S wild-type males were fed 1% fructose solutions containing concentrations of NQ ranging form 2.08 to 20.8 μg/mL for 72 hours. Twenty-five P_1 males surviving the approximate LC_{50} (actual dose was not reported) were individually mated with three virgin Basc females on days 1, 4, 6, and 8 postexposure. This mating sequence, therefore, represented the sampling of germ cells over the entire period of spermatogenesis. A maximum of 25 F_1 females from each culture of each brood were pair-mated to their brothers, and the progeny (F_2) were scored for lethal mutations. Confirmation of a lethal mutation was obtained by conducting an F_3 cross of each culture scored as a lethal mutation. Results indicated that there were no significant increases in the percent lethal mutations for individual broods or the combined total percentage for all four broods. Although the study was properly conducted, the failure to report the actual dose compromises the utility of these data in contributing to a risk assessment evaluation of NQ.

In a survey study for chromosome aberrations, NQ was reported to be a clastogen for Chinese hamster fibroblast (lung) cells when incubated in vitro at 4.0×10^{-4} molar, using DMSO as a solvent (Ishidate and Odashima, 1977). Aberrant cells treated with NQ were reported to display chromatid or chromosomal breaks, translocation, and chromatid gaps.

However, the study methodology and results have been questioned by the reviewers. Large doses of the compound were required to reach the maximum effective dose, cytotoxicity was not reported, and the incidence of chromatid gaps was included in the percentage calculation of aberrant cells. Equivalent doses reported as maximum effective dose levels (mg/mL and 10^{-4} M) did not correspond and were difficult to interpret.

Korolev et al. (1980) found no increase in the number of chromosome aberrations in bone marrow cells when animals were dosed with 0.5, 0.05, or 0.005 mg NQ/kg.

The potential of seven nonactivated (0.01 to 4.0 mg/mL) and seven S9-activated (0.01 to 3.9 mg/mL) doses of NQ to induce sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells was investigated by Harbell et al. (1988). Selection of the high dose was based on the solubility properties of NQ in aqueous medium. Cytotoxicity was apparent only in the absence of S9-activation (2.0 and 4.0 mg/mL); however, NQ did not significantly increase the frequency of SCEs either with or without S9 activation

NQ at a concentration of 10 mg/plate failed to induce DNA damage/repair in DNA-repair deficient E. coli strain P3478, PoL A^- (McGregor et al., 1980; Kenyon, 1982). Mitotic recombination was tested using cultures of the yeast Saccharomyces cerevisiae; no recombinogenic activity attributable to NQ was found (McGregor et al., 1980).

Brusick and Matheson (1978) evaluated the genotoxic potential of nitroguanidine in a series of in vitro and in vivo genetic toxicology assays. The results were as follows.

Gene Mutation--Nitroguanidine (NQ) assayed over five concentrations (0.1 to 500 μg/plate) in the absence and presence of exogenous metabolic activation derived from Aroclor 1254-induced rat liver (S9) was neither cytotoxic nor mutagenic in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, or TA100. The lack of a cytotoxic response at the highest dose, however, precludes acceptance of these data as valid evidence of a negative response.

The potential of NQ to induce forward mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells was also investigated. Five concentrations (1.25 to 5 mg/mL) were tested with and without an uninduced mouse liver microsome preparation. Results indicated that the test material was assayed to a cytotoxic level (5 mg/mL/+/-S9) with no indication of a reproducible or dose-related mutagenic response. However, the use of an uninduced mouse liver S9 preparation for testing of chemicals of unknown mutagenicity is not considered to be an acceptable practice.

Chromosome Aberrations--Germinal Cells -- As part of the overall investigation of NQ, Brusick and Matheson (1978) performed dominant lethal assays in two rodent species. Male mice or rats (10/group) received oral gavage administration of 0.2, 0.67, or 2.0 g NQ/kg for 5 consecutive days and were sequentially mated with unexposed virgin females (20/group/mating interval) for 7 weeks. In the experiment with mice, no clinical signs of toxicity were reported; NQ had no adverse effect on reproductive performance or relevant dominant lethal parameters. It was concluded that NQ assayed to an acceptably high dose did not cause a clastogenic response in male mouse germinal cells sampled over a 7-week period. Although it is recommended that the mouse dominant lethal assay be performed over an 8-week period, the study is acceptable for all stages of the spermatogenic cycle, except stem cells.

No definitive conclusions can be reached regarding the rat dominant lethal assay conducted with NQ because of the low fertility indices (FI) in both concurrent control and test groups at all mating intervals. Concurrent control FIs ranged from a low of 0.1 (2 of 20 pregnant) to a high of 0.65 (13 of 20 pregnant). The lack of fecundity in all groups may have been related to the age of the rats at study initiation (10 weeks); the generally accepted earliest breeding age for rats is 14 weeks. It was also noted that historical background FIs were lower than the expected rate ($\geq$0.8) for sexually mature rats. Since the crucial parameter in dominant lethal assays is the number of pregnant females per dose per mating interval, the

sample sizes resulting from the low FIs were insufficient to provide assurance that the statistical power was adequate to detect a doubling of the spontaneous background frequency of dominant lethal mutations.

DNA Damage/Repair--NQ (0.1 to 500 μg/plate/+/- rat S9 activation) was reported to be negative in the *Saccharomyces cerevisiae* D4 mitotic gene conversion assay. However, the study was compromised by the lack of cytotoxicity at the highest dose and suboptimal assay conditions (use of stationary rather than actively growing cells, exposure and cultivation of the yeast cells at 37°C rather than 30°C, and lack of assay sensitivity to detect the S9-activated positive control).

Similarly, the unscheduled DNA synthesis (UDS) assay in human embryonic lung WI-38 cells conducted with a concentration range of 0.1 to 5.0 mg NQ/mL was compromised. NQ was neither cytotoxic nor genotoxic; however, the length of exposure of the cells to the test material (1.5 hours) may have been too short to allow test material interaction with genetic material; a 4-hour exposure period is generally recommended for this cell line. Additionally, the use of an uninduced mouse liver S9 tissue homogenate is not recommended for testing unknown compounds in the WI-38 UDS assay.

7. Other Effects

The toxicity of NQ to freshwater fish, invertebrates, and algae was uniformly low; less than 50% of the organisms exposed to NQ were killed at concentrations up to the solubility limit of NQ in water (from 1,700 mg/L at 12°C to 3,000 mg/L at 22°C) (Van der Schalie, 1985). Little difference between acute and chronic toxicity was found in studies conducted with rainbow trout. Based on these results, NQ presents little hazard to aquatic organisms except at concentrations approaching its solubility limit.

Under laboratory conditions, photolysis of NQ with ultraviolet light increased its acute toxicity for one species of freshwater fish (fathead

minnows, Pimephales promelas), algae (Selenastrum capricornutum), and invertebrate (Daphnia magna) by factors of 66 to 115 (Van der Schalie, 1985). This indicates that the toxicity of NQ-containing wastewaters can increase significantly if the water is left in holding ponds exposed to sunlight. Aging of photolyzed NQ for 72 hours had little effect on its toxicity. No definitive information is available on the photolysis of NQ in natural waters. The importance of the increased toxicity of photolyzed NQ depends on the rate of photolysis and on whether such photolysis results in reaction products as toxic as those created under laboratory conditions.

Methemoglobinemia is not reported in humans or animals exposed to NQ or its wastewater effluent. Nitrite and nitrate are NQ photolytic products (Spanggord et al., 1987) and nitrate was found in NQ wastewater effluent (American Cynamid, 1955); therefore, public health professionals should be aware of the potential for methemoglobinemia. Appropriate regulatory requirments for controlling nitrites and nitrates in water should be consulted.

VII. HEALTH ADVISORY DEVELOPMENT

A. SUMMARY OF HEALTH EFFECTS DATA

No studies on the health effects of NQ in humans have been reported in the literature.

Acute toxicity studies by Korolev et al. (1980) indicated oral LD_{50} values of 3.85 g/kg in mice and 10.20 g/kg in rats. Females were more sensitive to seizure induction; convulsions were exhibited in female mice dosed at 3.98 to 6.31 g/kg (Hiatt et al., 1988a,b). Based on increased water consumption, and on decreased serum electrolytes in male and female rats fed 1,000 mg/kg/day in a 14-day subacute feeding study, NQ was considered to be an osmotic diuretic and was found to be excreted unchanged in the urine (Morgan et al., 1988a). Reduced heart weights were also seen in females at this same dose.

Subchronic 90-day feeding studies in mice and rats indicate increased water consumption in males and females fed 1,000 mg/kg/day. In rats, mean body weights were decreased for 9 of 13 study weeks in females fed NQ at 1,000 mg/kg/day (Morgan et al., 1988b). A nonsignificant reduction in absolute and relative heart weights was exhibited in male rats in a dose-related manner. In male mice, reduced absolute and relative heart weights were seen at a dose level of 1,000 mg/kg/day (Frost et al., 1988).

NQ was found to cause significant changes in hematologic indices and the enzyme-generating function of the liver at dose levels of 0.05 and 0.5 mg/kg/day in a chronic toxicity study (Korolev et al., 1980). No further information was reported. No carcinogenicity studies on NQ are currently available. NQ is classified as Group D: Not Classified as to Human Carcinogenicity.

NQ was not found to be cytotoxic or mutagenic in microbial systems (Brusick and Matheson, 1978; Ishidate and Odashima, 1977; Kaplan et al., 1982; McGregor et al., 1980). However, the results are inconclusive because of the absence of reported data, the inability to demonstrate test

material interaction with the target cell (i.e., cytotoxicity), or the performance of assays under suboptimal conditions. NQ gave negative results in a dominant-lethal assay conducted in mice (Brusick and Matheson, 1978). A dominant lethal assay conducted in rats (Brusick and Matheson, 1978) was considered to be unacceptable. NQ gave negative results in a mitotic gene conversion assay and in an unscheduled DNA synthesis assay (Brusick and Matheson, 1978); however, assay conditions were considered to be suboptimal and unacceptable.

No gonadotoxic or mutagenic effects were found after dosing with 20, 100, or 500 mg NQ/kg for 40 days or 0.005, 0.05, 0.5 mg NQ/kg during a chronic toxicity study (Korolev et al., 1980). No other reproductive effect was evaluated. Maternal toxicity (as evidenced by deaths in 3/24 dams, convulsions, CNS effects, and decreased body weights and food consumption) and fetal toxicity/developmental effects (as evidenced by the increased incidence of resorptions, decreased size and weight of pups, and increased incidence of skeletal variations) was seen in rats at 1,000 mg NQ/kg/day (Coppes et al., 1988a); NQ was not found to be teratogenic. Developmental effects (as evidenced by the increased incidence of resorptions and increased incidence of skeletal variations) were found in rabbits dosed at 100, 316, and 1,000 mg/kg/day (Coppes et al., 1988b). However, the percent of resorptions/litter for dams administered 100 or 316 mg/kg/day was considered to be within the range of that of strain-matched historical controls; the percent of resorptions exhibited in the study control group was considered to be unusually low when compared with this range in historical controls. Nevertheless, this increased incidence in percent of resorptions/litter is considered to be equivocal evidence for developmental toxicity of NQ in rabbits.

B. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years), and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic endpoint of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(NOAEL \text{ or } LOAEL)\ (bw)}{(UF)\ (____\ L/day)} = _____ mg/L\ (_____\ \mu g/L)$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg bw/day).

bw = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100, or 1,000), in accordance with NAS/ODW guidelines.

L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

In a teratology study in New Zealand White rabbits, the percent of resorptions/litter was significantly increased in dams administered 100, 316, or 1,000 mg NQ/kg/day; however, the percent of resorptions/litter for dams administered 100 or 316 mg/kg/day was considered to be within the range of that of strain-matched historical controls (Coppes et al., 1988b). Nevertheless, this increased incidence in percent of resorptions/litter is considered to be equivocal evidence for developmental toxicity of NQ in rabbits. Based on this study and in accordance with USEPA guidelines, an additional uncertainty factor of 3 is incorporated into the derivation of the One-day, Ten-day, Longer-term, and Lifetime HAs for NQ (USEPA, 1990).

1. <u>One-day Health Advisory</u>

Acute studies on NQ were not judged to be suitable for determining a One-day HA value; it is recommended that the Ten-day HA for a 10-kg child (11 mg/L) be used as a conservative estimate for the One-day HA value.

2. <u>Ten-day Health Advisory</u>

In a 14-day study in Sprague-Dawley rats, increased water consumption and decreased electrolytes occurred in males and females, and reduced heart weights occurred in females receiving NQ in the diet at 1,000 mg/kg/day. No significant changes in electrolytes or organ weights

occurred at 316 mg/kg/day (Morgan et al., 1988a). Based on this study, the LOAEL is 1,000 mg/kg/day, and the NOAEL is 316 mg/kg/day.

The Ten-day HA is based on the calculation for a 10-kg child. This calculation is as follows:

$$\text{Ten-day Ha} = \frac{(316\ \text{mg/kg/day})\ (10\ \text{kg})}{(1\ \text{L/day})\ (100)\ (3)} = 10.5\ \text{mg/L}$$

(rounded off to 11 mg/L or 11,000 μg/L)

where:

316 mg/kg/day = NOAEL, based on increased water consumption and decreased electrolytes in male and female rats, and reduced heart weights in female rats following a 14-day dietary dosing at 1,000 mg/kg/day.

10 kg = assumed body weight of a child.

1 L/day = assumed water consumption of a 10-kg child.

100 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a NOAEL from an animal study.

3 = additional uncertainty factor, based on equivocal evidence of developmental toxicity in rabbits, which is of critical concern in determining the appropriate HA for a child.

3. Longer-term Health Advisory

The 90-day feeding study in rats by Morgan et al. (1988b) will be used to derive the Longer-term HA. Decreased body weights for 9 of 13 study weeks occurred in female rats receiving NQ in the diet at 1,000 mg/kg/day. Water consumption was increased in male and female rats receiving the same dose. Body and organ weight changes did not occur at 316 mg/kg/day. No compound-related histopathological lesions were observed in dosed rats. Based on this study, the LOAEL is 1,000 mg/kg/day, and the NOAEL is 316 mg/kg/day. Results of the 90-day feeding study in mice (Frost et al., 1988) were less definitive. Reduced heart weights occurred in males administered NQ in the diet at 1,000 mg/kg/day. Water consumption was increased in male and female mice at the same dose level. No remarkable gross or histopathologic changes were observed. This study supports the level of toxicity found in the study with rats.

The Longer-term HA for a 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(316\ \text{mg/kg/day})\ (10\ \text{kg})}{(1\ \text{L/day})\ (100)\ (3)} = 10.5\ \text{mg/L}$$

(rounded off to 11 mg/L or 11,000 μg/L)

where:

316 mg/kg/day = NOAEL, based on decreased body weight and increased brain-to-body weight ratios in female rats, and increased water consumption in male and female rats following 90-day dietary dosing at 1,000 mg/kg/day.

10 kg = assumed body weight of a child.

1 L/day = assumed water consumption of a 10-kg child.

100 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a NOAEL from an animal study.

3 = additional uncertainty factor, based on equivocal evidence of developmental toxicity in rabbits, which is of critical concern in determining the appropriate HA for a child.

The Longer-term HA for a 70-kg adult is as follows:

$$\text{Longer-term HA} = \frac{(316\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \text{L/day})\ (100)\ (3)} = 36.9\ \text{mg/L}$$

(rounded off to 37 mg/L or 37,000 μg/L)

where:

316 mg/kg/day = NOAEL, based on increased body weight and increased brain-to-body weight ratios in female rats and increased water consumption in male and female rats following 90-day dietary dosing at 1,000 mg/kg/day.

70 kg = assumed body weight of an adult.

2 L/day = assumed daily water consumption of a 70-kg adult.

100 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a NOAEL from an animal study.

3 = additional uncertainty factor, based on equivocal evidence of developmental toxicity in rabbits.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered predictive of noncarcinogenic adverse health effects over a lifetime exposure. The

Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The lifetime HA is determined in step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water may be based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in assessing the risks associated with lifetime exposure to this chemical.

No acceptable long-term toxicity studies for NQ were found in the literature. Therefore, the 90-day feeding study in rats (Morgan et al., 1988b) discussed above will be used to derive a Reference Dose for NQ.

NQ was administered in the diet to male and female Sprague-Dawley rats at concentrations of 0, 100, 316, or 1,000 mg/kg/day for 90 days (Morgan et al., 1988b). Based on body weight changes in females, the LOAEL was 1,000 mg/kg/day, and the NOAEL was 316 mg/kg/day. No compound-related histopathological lesions were observed in dosed rats. A NOAEL of 316 mg/kg/day was reported for females based on the absence of body weight changes.

The DWEL is derived as follows:

Step 1: Determination of the Reference Dose (RfD)

$$RfD = \frac{(316\ mg/kg/day)}{(1{,}000)\ (3)} = 0.105\ mg/kg/day$$

where:

316 mg/kg/day = NOAEL, based on the absence of body and organ weight changes in female rats.

1,000 = uncertainty factor, chosen in accordance with NAS/ODW guidelines (10 x for intraspecies variability, 10 x for interspecies variability, and 10 x for use of a NOAEL from an animal study of less-than-lifetime duration).

3 = additional uncertainty factor based on equivocal evidence of developmental toxicity in rabbits.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$DWEL = \frac{(0.105\ mg/kg/day)\ (70\ kg)}{2\ L/day} = 3.675\ mg/L$$ (rounded off to 4.0 mg/L or 4,000 μg/L)

where:

0.105 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L/day = assumed daily water consumption of an adult.

Step 3: Determination of Lifetime Health Advisory

Lifetime HA = (3.675 mg/L) (0.2) = 0.735 mg/L (rounded off to 0.74 mg/L or 740 μg/L)

where:

3.675 mg/L = Drinking Water Equivalent Level (DWEL).

0.2 = assumed Relative Source Contribution (RSC) if actual exposure data are not available.

C. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Applying the criteria described in EPA's guidelines for assessment of carcinogenic risk (U.S. EPA, 1986), NQ is classified in Group D: Not Classified as to Human Carcinogenicity.

VIII. OTHER CRITERIA, GUIDANCE, AND STANDARDS

The American conference of Governmental Industrial Hygienists (ACGIH) and the Occupational Safety and Health Administration (OSHA) have not determined a Threshold-Limit-Value (TLV), Short-Term-Exposure-Limit (STEL), or Permissable-Exposure-Limit (PEL) for NQ. The U.S. Army has proposed an interim 8-hour workplace PEL of 4.0 mg/m^3 total dust (USAEHA, 1990). The exposure limit was derived to protect the health of adult workers potentially exposed to NQ in Army Ammunition Plants. The proposed limit is based upon the USEPA oral RfD for NQ modified for assumptions associated with inhalation exposure of particulates.

IX. ANALYTICAL METHODS

The preferred analytical techniques for determination of nitroguanidine in production wastewater is reversed-phase high-performance liquid chromatography (RP-HPLC), which has been found to be a sensitive, reproducible, and rapid method of quantitative estimation (Kenyon, 1982; Burrows et al., 1984). Nitrosoguanidine, cyanoguanidine, melamine, and ammeline, additional organic constituents of NQ production wastewater, may be analyzed by the same methodology. In addition, guanidine may be analyzed by ion chromatography, and cyanamine and urea may be analyzed by spectrophotometric methods.

NQ is not readily analyzed by gas chromatography because of its low volatility and solubility characteristics (Small and Rosenblatt, 1974). NQ dust in air samples may be analyzed spectrophotometrically or by titrimetric assay.

X. TREATMENT TECHNOLOGIES

A. MICROBIAL DEGRADATION OF NITROGUANIDINE

Kaplan et al. (1982) determined that NQ could be metabolically reduced to nitrosoguanidine in anaerobic continuous culture using acclimated microorganisms. Under the conditions of the study, no evidence was found for further microbial reduction of nitrosoguanidine (Williams and MacGillivray, 1987). Kenyon (1982) found that nitrosoguanidine could be reduced abiotically. No transformation of NQ occurred under aerobic conditions.

Recent microbial toxicity testing by the Polybac Corporation (cited in Williams and MacGillivray, 1987) indicated that the raw acidic NQ manufacture wastewater was toxic to biological populations. However, when pretreated with lime and heated to 70°C, 30% of the NQ in the wastewater was transformed to innocuous products by a biological submerged film reactor system.

Kaplan and Kaplan (1985) studied the degradation of NQ in soil using continuous flow soil columns. NQ was found to be anaerobically biodegradable on a short-term basis if sufficient supplemental carbon was provided in the wastewater for co-metabolic needs. The carbon requirement was dependent on the concentration of NQ in the wastewater, flow rate, soil type, hydraulic loading of the soil, and presence of other organics or inorganics. The authors determined that adequate monitoring of process waters, groundwater, and soil is therefore necessary. The degradation of 150 mg NQ/L required 0.5 to 1.0% glucose, representing a carbon to nitrogen ratio of approximately 34:1 and 68:1, respectively. The primary product formed during the biodegradation of NQ in soil was ammonia, with only trace concentrations of nitrosoguanidine. Without supplemental carbon, NQ will not degrade and will leach directly into the groundwater.

Williams and MacGillivray (1987) studied the use of continuous flow and perfusion soil columns as methods of NQ wastewater component removal and found them to be only partially effective.

In mineralization studies, in which a mineralization rate potential was established, NQ was poorly transformed with less than 15% of the parent compound evolved as CO_2. Addition of carbon supplements, nutrients, acclimated microorganisms, or incubation under aerobic or anaerobic conditions did not significantly alter mineralization of NQ. These studies indicated that NQ would probably be poorly removed in a land treatment system and could potentially contaminate groundwater. Inorganic constituents of NQ wastewater (e.g., nitrate and sulfate) could cause further groundwater contamination (Williams and MacGillivray, 1987).

B. ULTRAVIOLET IRRADIATION

Ultraviolet irradiation has been suggested by Kaplan and Kaplan (1985) as an alternative in alleviating pollution hazards associated with nitroguanidine-laden waste streams. Noss and Chyrek (1984) have reported that aqueous NQ degrades in strong ultraviolet (UV) light with a half-life of approximately 20 hours. Butler (1983, as cited in Noss and Chyrek, 1984) has indicated that 98% of an aqueous solution containing 1,100 mg NQ/L was degraded by a photolytic process in 4 to 5 hours. Concentrations of 0.1% or greater hydrogen peroxide were found to inhibit photolytic destruction, although concentrations of 0.01% were found to enhance photolytic activity and radical formation. Irradiation of 100-mg NQ/L solutions for 1 hour produced a 60% loss of NQ. The rate of degradation was not pH dependent.

Nitrosoguanidine was found to be produced as an intermediate during NQ degradation with UV light. The UV light subsequently degraded nitrosoguanidine to guanidine-nitrogen, nitrate-nitrogen, and nitrite-nitrogen. The end products of ultraviolet photolysis were dependent upon the wastewater pH (Noss and Chyrek, 1984). Differences in the amount of recoverable total nitrogen at acidic and alkaline pH values were associated with the production of guanidine.

The rate of ultraviolet photolysis of NQ was independent of pH, but the production of guanidine occurred only below pH 11. Guanidine formation from photolysis of NQ must be considered when effluent limits for NQ and guanidine are set. The use of hydrogen peroxide or ozone in

conjunction with UV light did not increase the rate of NQ destruction (Noss and Chyrek, 1984). Fields et al. (1985) reported the results of an ultraviolet irradiation study in which NQ in crystallizer condensate was reduced from 15.5 mg/L to less than 1 mg/L in 27 minutes.

C. OTHER METHODOLOGIES

The adsorption of NQ on activated carbon (Calgon FS-300 GAC) reduced the NQ concentration in crystallizer condensate from 15.5 mg/L to less than 1 mg/L, giving a carbon capacity of approximately 0.045 g NQ/g carbon (Fields et al., 1985).

NQ can be effectively reduced by contact with ion exchange resin (Okamoto, 1978). The cation exchange of the Gu^+ (guanidinium ion), using duolite C-20 NA^+ strong acid resin, reduced the Gu^+ concentration in stripped evaporator condensate from 14.5 to 1 mg/L, giving an average resin capacity of 0.13 eq/L resin (Fields et al., 1985). NQ has been found to be unaffected by treatment with quaternary amine surfactant (Okamoto, 1978).

NQ has been found to be effectively decomposed with sodium hydroxide in combination with sodium sulfide, the decomposition rate being a function of the sulfide concentration added. However, the use of large amounts of sulfide proved to be undesirable (Smith et al., 1983a,b).

Additional studies are necessary to determine treatment technologies for a permanent NQ wastewater treatment facility.

XI. CONCLUSIONS

Based on decreased electrolytes, increased water consumption, and reduced heart weights in rats administered NQ in the diet for 14 days, and on eqivocal evidence for developmental toxicity in rabbits administered NQ by oral gavage for 12 days, the One-day and Ten-day HA for exposure in a 10-kg child has been determined to be 11 mg/L (11,000 μg/L). Based on decreased body weights, increased relative brain weights, reduced heart weights, and increased water consumption in rats and mice administered NQ in the diet for 90 days, and on equivocal evidence for developmental toxicity in rabbits, the Longer-term HA for exposure in a 10-kg child has been determined to be 11 mg/L (11,000 μg/L); the Longer-term HA for exposure in a 70-kg adult was determined to be 37 mg/L (37,000 μg/L). A Lifetime HA of 0.74 mg/L (740 μg/L) for a 70-kg adult is based on a Drinking Water Equivalent Level (DWEL) of 4.0 mg/L (4,000 μg/L). The DWEL is based on a NOAEL of 316 mg/kg/day; the NOAEL is based on the absence of body and organ weight changes in female rats fed NQ for 90 days.

Since no chronic toxicity or carcinogenicity studies with NQ are currently available, NQ is classified in Group D: Not Classified as to Human Carcinogenicity.

A comparison report "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for NQ" (Appendix A) summaries the scope of existing data reviewed for this HA. This comparison report delineates the areas where additional data and/or a clarification of existing data would be appropriate for a second HA.

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APPENDIX A

Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for NQ

DATA BASE DEVELOPMENT

A. OBJECTIVES

The objective of this document is to provide an evaluation of data deficiencies and/or problem areas encountered in the review process for nitroguanidine (NQ) and to make recommendations, as appropriate, for additional data base development. This document is presented as an independent analysis of the current status of NQ toxicology, as related to its possible presence in drinking water, and includes a summary of the background information used in development of the Health Advisory (HA). For greater detail on the toxicology of NQ, the Health Advisory on NQ should be consulted.

B. BACKGROUND

Nitroguanidine, a colorless, crystalline solid that may exist in two tautomeric forms (alpha, usually produced during manufacture of NQ, and beta), is used in military munitions formulations. NQ is soluble in water to 4.4 mg/L at 25°C and may be found in wastewater from NQ manufacturing and loading operations.

Nitroguanidine is rapidly absorbed through the gastrointestinal tract, rapidly enters the blood, and is quickly excreted unchanged in the urine. This passage is facilitated by the small molecular size of the compound. The route of administration does not affect the disposition of the compound. Of the [^{14}C]NQ orally administered to rats, 40 to 50% was found in the urine within 4 hours; approximately 95% of the administered radiolabel was recovered in the urine within 48 hours after dosing. Small amounts of radiolabel found in the feces (0.4 to 1.6%) were considered to be due to urine contamination. Labeled carbon dioxide, [^{14}C]CO_2, was not detected in expired air following administration of [^{14}C]NQ.

The health effects of NQ have not been reported in humans. The oral LD_{50} values of NQ in mice and rats are approximately 3.9 and 10.2 g/kg, respectively (Korolev et al., 1980). After a single oral dose, effects on

respiration, the gastrointestinal tract, and the central nervous system (CNS) were exhibited. Mice were more susceptible to CNS effects; females were most susceptible to convulsions at doses of 6.31, 5.01, and 3.98 g/kg (Hiatt et al., 1988a,b).

In a 14-day study, serum electrolytes were decreased and water consumption was increased in male and female rats fed 1,000 mg/kg/day. Reduced heart weights were also seen in females at the same dose level. No compound-related histopathological effects were observed. No significant changes in electrolytes or organ weights occurred at 316 mg/kg/day. NQ was considered to be an osmotic diuretic and was excreted unchanged in the urine (Morgan et al., 1988a).

In a 90-day feeding study in rats, NQ was administered in the diet at levels between 100 and 1,000 mg/kg/day. Mean body weights were decreased for 9 of 13 weeks in females fed NQ in the diet at 1,000 mg/kg/day. Water consumption was increased in males and females receiving the same dose. No compound-related histopathological lesions were observed in dosed rats (Morgan et al., 1988b). The No-Observed-Adverse-Effect Level (NOAEL) was 316 mg/kg/day. In a 90-day feeding study with mice, reduced heart weights and increased brain-to-body weight ratios occurred in males fed NQ in the diet at 1,000 mg/kg/day. Water consumption increased in males and females at the same dose. No compound-related histopathological effects were exhibited; no remarkable changes in organ weights occurred at 316 mg/kg/day (Frost et al., 1988).

NQ was found to cause significant changes (nature unspecified) in hematologic indices and in the enzyme-generating function of the liver at dose levels of 0.05 and 0.5 mg/kg/day in a chronic toxicity study (Korolev et al., 1980). However, results are inconclusive because of the absence of any other reported data. No carcinogenicity studies on NQ are currently available.

The <u>in vitro</u> and <u>in vivo</u> genetic toxicology assays conducted with NQ were uniformly negative. With the exception of a single mouse dominant lethal assay, however, the various studies with NQ were flawed either by an inability to demonstrate test material interaction with the target cell

(i.e., cytotoxicity) or the performance of assays under suboptimal conditions. It is, therefore, concluded that further study of all major genetic endpoints is required before an assessment of the genotoxic potential, if any, of NQ is possible (Brusick and Matheson, 1978; Ishidate et al., 1977, as cited in Kenyon, 1982; Kaplan, 1982, as cited in Kenyon, 1982; McGregor et al., 1980).

No reproductive effects were found after dosing with 20 to 500 mg NQ/kg for 40 days; however, results are inconclusive because of the absence of any other reported data (Korolev et al., 1980). Developmental effects (increased incidence of resorptions, decreased pup size and weight, and increased incidence of skeletal variations) were seen at 1,000 mg NQ/kg/day in rats and rabbits. Nitroguanidine caused maternal toxicity in rats at 1,000 mg/kg/day but was not found to be teratogenic (Coppes et al., 1988a). Equivocal evidence was found for developmental toxicity in rabbits at 100, 316, and 1,000 mg/kg/day (Coppes et al., 1988b).

The methods for analysis of NQ in wastewater and the methods for treating NQ-contaminated water appear to be adequate.

C. DISCUSSION

Available data on the pharmacokinetics, health effects, analyses, and treatment of NQ have been reviewed.

Pharmacokinetic data on orally administered NQ indicate rapid absorption through the gastrointestinal tract with little potential for bioaccumulation in tissue; NQ is excreted primarily in the urine with a minimal amount of metabolic conversion. Further studies in animals are unlikely to yield additional data pertinent to the development of HA values.

Acute toxicity studies include oral LD_{50}s in mice, rats, and guinea pigs. The median lethal dose (MLD) of NQ was determined in mice and rats.

Subacute (14-day) and subchronic (90-day) studies were available for Sprague-Dawley rats and ICR mice. These studies appeared to be adequate;

additional subacute studies in doses greater than 1,000 mg NQ/kg/day in mice may specify CNS effects and may be used to address emergency exposures for the One-day HA.

No adequate chronic toxicity or carcinogenicity studies on NQ were available; the Drinking Water Equivalent Level (DWEL) value was derived from a 90-day study with rats. These data gaps should be filled.

Study deficiencies of existing genotoxicity data on NQ require that further study of all major genetic endpoints be conducted prior to a final assessment of the genotoxic potential of NQ.

No adequate studies on the reproductive effects of NQ were available.

D. CONCLUSIONS/RECOMMENDATIONS

The following conclusions/recommendations are based upon the above discussion:

1. The available studies on NQ toxicity are limited for development of Health Advisories useful in dealing with the potential contaminants of drinking water.

2. It is recommended that chronic toxicity/carcinogenicity studies be performed in rats and mice.

3. It is recommended that adequate reproduction studies be performed in males and females of at least one rodent species.

4. It is recommended that adequate genotoxicity studies be performed in microbial and nonmicrobial cell systems.

5. It is recommended that NQ human health effects be studied. Potential study groups are people occupationally exposed and people who drink water containing measurable quantities of NQ.

The recommended order of completion for these studies is as follows: chronic toxicity/carcinogenicity, genotoxicity, reproduction, delayed neuropathy and human health effects.

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APPENDIX B

Adjunct Developmental Toxicity Data

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULE-T

10 May 1989

Dr. William R. Hartley
US Environmental Protection Agency
Office of Drinking Water (WH550D)
401 M St. SW
Washington DC 20460

Re: Developmental Toxicity of Nitroguanidine in Rabbits

Dear Dr. Hartley:

At the request of Dr. Reddy at USABRDL, Ms. Coppes and I have reviewed the data and conclusions in Toxicology Series Report 184 "Developmental Toxicity Potential of Nitroguanidine in Rabbits" (LAIR Institute Report No. 298) with respect to the number of resorptions/litter and percentage resorptions. Our review confirmed that the data as presented in the report are accurate; but, our review also indicated that the conclusions as stated in the report could be interpreted more strongly than was intended.

There was a statistical increase in the number of litters with resorptions in the 100 and 1000 mg/kg dose groups (Table 1) and in the percentage resorptions (resorptions/implants X 100%) in all (100, 316, and 1000 mg/kg) dose groups (Table 5) versus the control group. However, the "control" values for resorptions in this study were lower than published reports for NZW rabbits. Therefore, the experimental values (although within the published range of normal values for control animals) were identified as statistically different from the study control. While there is the possibility that the data may be an accurate assessment of the effect of nitroguanidine on embryonic development in the rabbit, it is more likely a statistical aberration. Thus, a more accurate statement of our conclusions concerning the rabbit teratology study would be that the resorption data provide equivocal evidence that nitroguanidine is a developmental toxicant in the rabbit.

If you have any questions, we may be contacted telephonically at 415/561-2963.

Sincerely,

Valerie G. Coppes

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Table B-1. Comparison of Control Resorption Data and IAIR Study Resorption Data in Rabbits

Control Resorption Data for Rabbits From the Recent Literature[a]

Ref. No.	N[b]	C. lutea[c]	Implants[c]	Resorptions[c]	% Resorptions
1	22	10.9	9.6	1.3	13.5
2	18	11	8	0.4	6
3	10	9.9	5.2	0.7	13.5
4	25	10	7	0.4	6
5	21	9	7	1.1	14
6	21	7.5	7.5	1.2	16
7	22	11.3	9.0	0.3[d]	3.3
8	12	11.7	8.2	1.1	9.2
9	20[e]	11.6	10.1	1.6	15.2
		IAIR Nitroguanidine Data			
Control	13	10.3	9.5	0.2	2.2
100 mg/kg	15	11.2	10.2	0.9	9.3
316 mg/kg	15	11.1	9.3	0.5	6.6
1000 mg/kg	11	11.0	9.6	0.9	17.9[f]

[a]Data reported from literature with exception of resorptions/litter, which were calculated from reported data.
[b]Dams with viable litters.
[c]Per litter.
[d]Only early resorptions were reported.
[e]Data nonspecific.
[f]Value of % resorptions in high-dose rabbits was calculated by the reviewers to be 17.9; the % resorptions reported was 9.7.

PUBLISHED LITERATURE REVIEWED FOR RABBIT CONTROL VALUES

1. Evaluation of the Developmental Toxicity of Ethylene Gylcol Monohexyl Ether Vapor in Fischer 344 Rats and New Zealand White Rabbits. Tyl et al. Fund. Appl. Toxicol. 12:269-280, 1989.

2. Teratologic Evaluation of a Polybromodiphenyl Oxide Mixture in New Zealand White Rabbits Following Oral Exposure. Breslin et al. Fund. Appl. Toxicol. 12:151-157, 1989.

3. Developmental Toxicology Investigation of Tellurium. Johnson et al. Fund. Appl. Toxicol. 11:691-702, 1988.

4. Teratologic Evaluation of Orally Administered Nitrapyrin in Rats and Rabbits. Berdasco et al. Fund. Appl. Toxicol. 11:464-471, 1988.

5. Teratological Evaluation of Diglycidyl Ether of Bisphenol A (DGEBPA) in New Zealand White Rabbits Following Dermal Exposure. Breslin et al. Fund. Appl. Toxicol. 10:736-743, 1988.

6. Evaluation of the Developmental Toxicity of β(3,4-Epoxy-cyclohexyl-ethytrimethoxysilane in Fischer 344 Rats and New Zealand White Rabbits. Tyl et al. Fund. Appl. Toxicol. 10:439-452, 1988.

7. Developmental Toxicity Evaluation of Inhaled 2-Ethoxyethanol Acetate in Fischer 344 Rats and New Zealand White Rabbits. Tyl et al. Fund. Appl. Toxicol. 10:20-39, 1989.

8. Teratologic Studies on Alcide Allay Gel in Rabbits. Abdel-Rahman et al. J. Appl. Toxicol. 7:161-166, 1987.

9. Developmental Toxicity Studies of Caprolactam in the Rat and Rabbit. Gad et al. J. Appl. Toxicol. 7:317-326, 1987.

APPENDIX C

Toxicity of Associated Compounds: Guanidine Hydrochloride and Guanidine Nitrate

TOXICITY OF ASSOCIATED COMPOUNDS: GUANIDINE HYDROCHLORIDE AND GUANIDINE NITRATE

A. HUMANS

No studies on the health effects of guanidine hydrochloride and guanidine nitrate in humans have been found in the literature.

B. ANIMAL EXPERIMENTS

1. Short-term Exposure

a. Acute

1) Guanidine hydrochloride

Table C-1 lists acute toxicity values for nitroguanidine analogs. Guanidine hydrochloride was found to be "slightly to moderately toxic" in Sprague-Dawley rats, with an LD_{50} of 556.5 ± 29.7 mg/kg in males and 474.6 ± 35.3 mg/kg in females (Morgan et al., 1985). A total of 92% of the deaths occurred between 4 and 26 hours after a single dose was administered by oral intubation (9/9 males and females dosed at 775 mg/kg, 3/9 males and 4/7 females dosed at 600 mg/kg, 2/9 males and 4/9 females dosed at 464 mg/kg, and 2/8 females doses at 360 mg/kg). Three additional animals (two males administered 600 mg/kg and one female administered 464 mg/kg) were found dead on day 2. Clinical signs of hyperactivity and irritability 2 hours after dosing progressed to central nervous system-neuromuscular disturbances (increased startle reflex, hyperactivity, tremors, twitching, depressed grasping and righting reflexes, and disorientation) and gastrointestinal tract symptoms (increased salivation, hunched posture, and diarrhea) within 4 hours of dosing in all test groups. Rough hair coats and irregular rates and patterns of respiration were found sporadically among these guanidine hydrochloride-treated rats. Mean body weights were comparable between dosed and control males and females. Marked erythema and edema of the digestive system were found at necropsy in the animals whose deaths were reported to be compound related.

Table C-1. Acute LD_{50} Values for Nitroguanidine Analogs in Laboratory Animals

Analog	Species	Strain	Sex	Route	Vehicle	LD_{50} (g/kg)	Reference
GuHCL	Rat	Sprague-Dawley	M	oral	Water	0.56	Morgan et al. (1985)
			F	oral	Water	0.47	
GuN	Rat		M	oral	Water	1.26	American Cyanamid (1985)
GuN	Rat	Sprague-Dawley	M	oral	Methylcellulose/Tween-80	0.99 (MLD)	Hiatt et al. (1988b)
			F	oral		0.73 (MLD)	
	Mouse	ICR	M		Methylcellulose/Tween-80	1.11 (MLD)	
			F			1.02 (MLD)	

MLD = Median lethal dose.

2) Guanidine nitrate

The oral LD_{50} of guanidine nitrate administered to male albino rats was found to be 1.26 g/kg (American Cyanamid, 1955). It was reported that animals receiving doses of 2.15 and 4.64 g/kg exhibited exophthalmos, excessive salivation, labored respiration, diarrhea, and depressed righting and placement reflexes within 30 minutes of dosing. Deaths (number and associated doses not reported) occurring within 24 hours were preceded by mild clonic convulsions and coma. Necropsy of these animals revealed irritation of the gastrointestinal tract, hemorrhage of the lungs, and congestion of the kidneys and adrenals. Other than transient depression, no gross symptoms were reported at doses equal to or less than 1 g/kg. Hematuria was found in one of the four surviving males receiving 1 g/kg. Necropsy of this animal revealed mucoid material of the small intestine, congestion of the kidneys, and a yellow coloration of the liver, fat, testes, peritoneum, and small intestine.

Guanidine nitrate was administered by oral intubation as a suspension in 0.2% methylcellulose and 0.4% Tween-80 to Sprague-Dawley rats and ICR mice (Hiatt et al., 1988). The MLD was 990 mg/kg and 730 mg/kg for male and female rats, respectively, and 1,105 mg/kg and 1,017 mg/kg for male and female mice, respectively. The study authors reported that the MLD for the guanidine base alone was 491 mg/kg and 353 mg/kg in male and female rats, respectively; in mice, the corresponding values were 534 mg/kg in males and 493 mg/kg in females. Mortality data for GuN in rats and mice are presented in Tables C-2 and C-3. Clinical signs of toxicity produced by GuN were primarily associated with effects on the CNS and the neuromuscular system (ataxia, hyperactivity, disorientation, tremors, muscle twitching, and excessive jumping) in rats and mice. Gastrointestinal effects were considered to be secondary. No histopathological lesions associated with the CNS or GI symptoms were observed. Respiratory tract lesions and pulmonary congestion were found in male and female mice that died following dosing with GuN.

Doses of 2.0 g GuN/kg applied once to the intact skin of five male and five female New Zealand White rabbits resulted in no deaths and no signs of systemic toxicity (Hiatt et al., 1988). However, dermal

Table C-2. Mortality of Sprague-Dawley Rats Dosed Orally With Guanidine Nitrate

Dose level (mg/kg)	Compound-related deaths/ Number dosed in group[a]	% Mortality
	Males	
0	0/5	0
311	0/10	0
826	3/10	30
1,000	3/8	38
1,210	7/8	88
1,420	9/10	90
	Females	
0	0/5	0
610	1/7	14
718	5/9	56
847	6/9	67
1,000	10/10	100
1,180	10/10	100
1,390	8/8	100

[a]Median lethal dose (95% confidence limits): males, 990 (793, 1,138) mg/kg; females, 730 (641, 799) mg/kg.

SOURCE: Hiatt et al. (1988).

Table C-3. Mortality of ICR Mice Dosed Orally With Guanidine Nitrate

Dose level (mg/kg)	Compound-related deaths/ Number dosed in groups[a]	% Mortality
	Males	
0	0/5	0
708	0/10	0
891	3/10	30
1121	5/10	50
1410	9/10	90
1780	9/10	90
	Females	
0	0/5	0
708	2/10	20
891	9/14	64
1121	9/15	60
1410	6/10	60
1780	5/7	71

[a]Median lethal dose (95% confidence limits): males, 1,105 (957, 1,276) mg/kg; females, 1,017 (706, 1,465) mg/kg.

SOURCE: Hiatt et al. (1988).

irritation and inflammation were observed on all dosed animals; necrosis and eschar formation were observed in 1/10 rabbits.

b. Primary irritation and dermal sensitization

1) Guanidine hydrochloride

Using the modified Draize method for skin irritation, a paste of 0.5 GuHCL was applied directly to the shaved intact and abraded skin of three male and three female New Zealand White rabbits for 24 hours (Morgan et al., 1986b). Grading of the dermal reaction was performed 1, 2, 3, 7, and 14 days after application. GuHCL, classified as a severe dermal irritant, produced moderate to marked erythema followed by necrosis, eschar formation, and sloughing of the skin at both intact and abraded sites. Eschar formation occurred by day 3 at abraded sites and by week 2 at intact sites. Slight edema was found at the abraded sites in five of six (83%) rabbits at 24 hours; edema was very slight at intact sites.

Using the Buchler dermal sensitization method, 0.5 mL GuHCL was applied as a 10% solution to the shaved skin of 10 male Hartley guinea pigs for 6 hours once each week for 3 consecutive weeks during the induction phase (Hiatt et al., 1986). The same application site was used for each induction dose; saline control, dinitrochlorobenzene (DNCB) positive control, and negative control groups were treated consecutively. Two weeks following the third induction dose, the test compound was applied to the induction site and to an untreated site to distinguish between reactions from repeated insult and sensitization. GuHCL showed no evidence that indicated dermal sensitization. A minor response was reported in 1 of 10 animals following the third induction; no response followed challenge.

2) Guanidine nitrate

An aqueous paste of 1.0, 2.15, 4.64, or 10 g GuN/kg was applied to the clipped skin of four male albino rabbits for 24 hours (American Cyanamid, 1955). A mild degree of skin irritation characterized by slight erythema, which persisted from 1 to 4 days, was reported. One death, due

to an intestinal infection, was considered to be unrelated to administration of the test compound.

A paste of 0.5 g GuN moistened with saline was applied to the intact and abraded skin of eight albino rabbits for 24 hours (Hiatt et al., 1988b). Inflammation of the intact skin was severe in six of eight animals, although only minimal edema was observed; necrosis with eschar formation occurred at these sites within the following 7 days, remaining through the 14-day observation period. No sloughing occurred. The abraded skin of all animals became necrotic with eschar formation within 7 days of dosing; these lesions spread beyond the treated area in six of eight animals. Edema was more severe in these animals.

Using the modified Draize method for eye irritation, 0.092 g (0.1 mL) of GuN, administered as a crystalline powder, was applied directly to the eye of six male New Zealand White rabbits (Morgan et al., 1986a; Hiatt et al., 1988). The contralateral eyes served as the untreated controls. Grading of ocular reactions was performed at 1 and 4 hours and at 1, 2, 3, 7, 14, and 21 days. Fluorescein dye was used for grading at 24 hours and at 7, 14, and 21 days. Guanidine nitrate was found to be a mild ocular irritant with corrosive properties. All rabbits exhibited conjunctival redness and chemosis of varying degrees. Of the six animals, four developed corneal erosions and ulceration, which persisted through day 21 in two animals. The nictitating membrane was sloughed in three rabbits. One animal developed vascularization of the cornea from day 14 to day 21; vascularization of the iris had been observed in this animal 1 hour after exposure.

No evidence of skin sensitization was exhibited in guinea pigs following three induction doses or a challenge dose of 10% (in 0.9% saline) GuN (Hiatt et al., 1988).

c. Subacute

1) Guanidine hydrochloride

No studies have been found in the literature.

2) Guanidine nitrate

Levels up to 0.2% GuN in the diets of male albino rats for 30 days caused no significant effects on survival, food intake, weight gain, or gross pathology (American Cyanamid, 1955).

2. Long-term Exposure

The only available information on effects associated with long-term exposure is a mutagenicity study with guanidine hydrochloride reported by Sano et al. (1985).

The mutagenic potential of guanidine hydrochloride was evaluated using the Ames Salmonella/mammalian microsome mutagenicity assay. Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to guanidine hydrochloride at concentrations of 0.0016, 0.008, 0.04, 0.2, 1, and 5 mg/plate (Sano et al., 1985). The assays were conducted with and without metabolic activation. Guanidine hydrochloride was nontoxic and nonmutagenic.

3. References

American Cyanamid. 1955. Studies Relating to Effluent Disposal of Nitroguanidine Manufacture. U.S. Army Ordnance Corps. Stamford Laboratories. Contract No. DAI-30-069-501-ORD-(P)-1220.

Hiatt GFS, Morgan EW, Korte DW. 1986. Dermal Sensitization Potential of Guanidine Hydrochloride in Guinea Pigs. Toxicology Series 84. Letterman Army Institute of Research, San Francisco, CA, to U.S. Army Medical Research and Development Command, Fort Detrick, MD. Technical Report No. 210. Contract No. AD A164 655.

Hiatt GFS, Morgan EW, Brown LD, Lewis CM, Johnson YC, Mullen L, Bauserman JW, Okerberg CV, Lallini LO, Korte DW. 1988. Acute Toxicology of Guanidine Nitrate and Nitroguanidine. Jannaf Safety and Environmental Protection Subcommittee Meeting. Chemical Propulsion Information Agency. Contract No. N 00024-85-C-5301.

Morgan EW, Sano SK, Korte DW. 1985. Acute Oral Toxicity (LD_{50}) of Guanidine Hydrochloride in Rabbits. Toxicology Series 77. Letterman Army Institute of Research, San Francisco, CA, to U.S. Army Medical Research and Development Command, Fort Detrick, MD. Technical Report No. 204. Contract No. AD-A165 747.

Morgan EW, Bauserman JW, Korte DW. 1986a. Primary Eye Irritation of Guanidine Nitrate in Male Rabbits. Toxicology Series 86. Letterman Army Institute of Research, San Francisco, CA, to U.S. Army Medical Research and Development Command, Fort Detrick, MD. Technical Report No. 212.

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Sano SK, Kellner TP, Korte DW. 1985. Mutagenic Potential of Guanidine Hydrochloride (TP028). Toxicology Series 80. Letterman Army Institute of Research, San Francisco, CA, to U.S. Army Medical Research and Development Command, Fort Detrick, MD. Technical Report No. 197.