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Provisional Peer Reviewed Toxicity Values for

Nitroguanidine
(CASRN 556-88-7)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

ACRONYMS AND ABBREVIATIONS

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR NITROGUANIDINE (CASRN 556-88-7)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

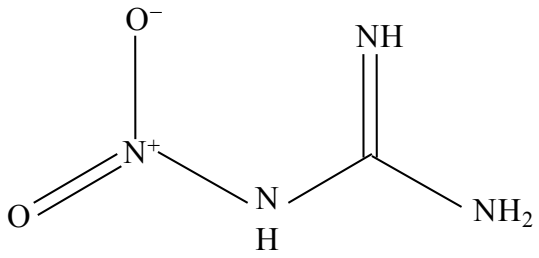
It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Nitroguanidine (CASRN 556-88-7) is a crystalline solid with explosive properties that have led to military use in munitions and propellants. It is also used as an intermediate in the synthesis of some pharmaceuticals (HSDB, 2007). The empirical formula for nitroguanidine is $CH_4N_4O_2$.



The U.S. Environmental Protection Agency (U.S. EPA)'s IRIS database (U.S. EPA, 1989) lists a chronic RfD of 0.1 mg/kg-day for nitroguanidine that references a Drinking Water Health Advisory document (U.S. EPA, 1990a). This RfD is also included in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). Both IRIS (U.S. EPA, 1990b) and the DWHA list (U.S. EPA, 2006) also include a Group D (not classifiable as to human carcinogenicity) cancer classification for nitroguanidine. IRIS does not include an RfC assessment for this chemical. Subchronic or chronic RfDs or RfCs, or cancer assessments for nitroguanidine are not listed in the HEAST (HEAST; U.S. EPA, 1997). The CARA list (U.S. EPA, 1991a, 1994) does not include nitroguanidine. No standards for occupational exposure to nitroguanidine have been established by the American Conference of Governmental Industrial Hygienists (ACGIH, 2007), the National Institute of Occupational Safety and Health (NIOSH, 2007) or the Occupational Safety and Health Administration (OSHA, 2006). The

ATSDR (ATSDR, 2007), International Agency for Research on Cancer (IARC, 2007), and the World Health Organization (WHO, 2007) have not published toxicological reviews on nitroguanidine.

Literature searches for studies relevant to the derivation of provisional toxicity values for nitroguanidine were conducted in MEDLINE, TOXLINE special, and DART/ETIC (1960s−July 2007); BIOSIS (August 2000−July 2007); TSCATS/TSCATS 2, CCRIS, GENETOX, HSDB, and RTECS (not date limited); and Current Contents (6 months prior to July 2007). An updated literature search (June 2007−November 2008) was conducted in PubMed.

REVIEW OF PERTINENT DATA

Human Studies

No studies investigating the effects of subchronic or chronic oral or inhalation exposure to nitroguanidine in humans were identified.

Animal Studies

Oral Exposure

Relevant information regarding the toxicity of nitroguanidine comes from a 90-day dietary study in rats (Morgan et al., 1988a), a 90-day dietary study in mice (Frost et al., 1988), a developmental toxicity study in rats (Coppes et al., 1988a), a developmental toxicity study in rabbits (Coppes et al., 1988b), and a 2-generation reproductive toxicity study in rats (Coppes et al., 1990). Information from these studies and from the Drinking Water Health Advisory Document for nitroguanidine (U.S. EPA, 1990a) is presented below.

Subchronic Studies—Groups of Sprague-Dawley rats (15/sex/dose level) were administered 0 (control), 100, 316, or 1000 mg/kg-day nitroguanidine (99.2−99.7% pure) in their diets for 90 days (Morgan et al., 1988a, unpublished report). Food and water consumption were measured on a weekly basis and the animals were observed twice daily for clinical signs. Body weights were recorded weekly and on the days of sacrifice. On day 45, 10 rats (5 of each sex) were sacrificed and submitted for necropsy. At the interim and terminal sacrifices, blood was collected for comprehensive hematology and clinical chemistry testing. At the terminal sacrifice, all major organs and tissues were weighted and processed for microscopic examination. There were no deaths or clinical signs attributable to dosing with nitroguanidine during the study. Food consumption was significantly reduced in the high-dose males on week 1 and in the high-dose females on weeks 5 and 6 relative to the controls ($p \leq 0.05$), and it is not clear whether this is due to food palatability issue. In general, water consumption was elevated in treated rats throughout the study, and the difference from the controls (25−35% at the high dose) achieved statistical significance for the mid- and high-dose rats. No significant differences in body weight were exhibited in the male dose groups. Body weights of high-dose females were significantly lower than controls at various times during the study and at termination, and the differences from

control values at the terminal sacrifice were about 8.8%. Results from clinical chemistry tests showed that there were no significant differences in serum electrolyte levels between the treated groups and the control groups for either sex. There were some significant differences with the controls in some serum biochemical parameters, e.g., increased cholesterol levels in high-dose males at interim and terminal sacrifice, decreased lactate dehydrogenase (LDH) activity and total protein levels in the mid-dose males at terminal sacrifice, and decreased triglyceride levels in low- and high-dose females at terminal sacrifice. However, all these values were within normal limits. Hematology tests were unremarkable. The mid-dose male rats showed a significant decrease in absolute adrenal gland weight as compared to controls at interim sacrifice, but not at the terminal sacrifice. There were no consistent, treatment-related changes in any organ weights (including the adrenal gland) or organ-to-body weight or organ-to-brain weight ratios in male rats. The females showed significantly decreased ovarian weights for all dose groups at interim sacrifice, but no significant changes in the ovarian-to-body weight or ovarian-to-brain weight ratios. The differences in ovarian weight do not appear to be dose related, and the same changes were not observed at the terminal sacrifice. A statistically significant decrease in brain weight at interim sacrifice (6%), and a significant increase in relative brain weight (9%) at terminal sacrifice were noted in high-dose females; although the latter effect could have been related, at least in part, to the reduced growth rate in high-dose females. Gross and microscopic examination of the tissues and organs did not reveal any treatment-related lesions, including the adrenal gland, ovaries, brain, and kidneys. Although there was a dose-related increase in water consumption in both sexes, it is considered a compensatory effect that may result in an accelerated excretion of the less soluble test compound compared to related urea which has been used as an osmotic diuretic. In addition, there were no kidney pathological changes and no evidence of disturbance of electrolyte levels in the serum. The difference in body weight between high-dose female rats and controls was not considered adverse because this change was less than 10% (a change commonly considered as an indication of reaching MTD), and the decreased weight gain could be attributed to reduced food consumption during the study. The significant decreases in ovarian weight were not considered adverse because several considerations: (1) they were not dose related; (2) they were only observed at the interim sacrifice (not at the terminal sacrifice); (3) there were no significant histo-pathological changes in the same organ in any of the treated dose groups. Based on these considerations, the high dose of 1000 g/kg-day can be defined as a freestanding NOAEL in this study.

In a similar study, groups of ICR mice (15/sex/dose-level) were administered 0 (control), 100, 316, or 1000 mg/kg-day nitroguanidine (the purity information was not available) in the diet for 90 days (Frost et al., 1988, unpublished report). The study protocol and endpoints evaluated were the same as in the rat study (Morgan et al., 1988a) summarized above. Treatment with nitroguanidine produced no mortality, and no clinical signs attributable to dosing with nitroguanidine were observed. Neither body weight gain nor food consumption was significantly affected by dosing with nitroguanidine. Water consumption was significantly increased in high-dose females during the second and third week of the study and in high-dose males throughout most of the study. Hematology values were not significantly affected. In male rats, the only significant changes in clinical chemistry values were increased aspartate amino-transferase (AST) activity in the high-dose group at interim sacrifice and increased uric acid in the mid-dose group at the terminal sacrifice. In female rats, the only significant clinical chemistry changes were increased albumin levels and albumin-globulin ratio in mid- and

high-dose groups at the interim sacrifice. However, all these changes remained within the respective normal ranges and did not exhibit consistent dose-response relationships. Organ-to-body-weight ratios and organ-to-brain-weight ratios were not significantly different from the controls, with the exception of an increase in relative brain weight in the high-dose males at interim sacrifice. Gross and microscopic examination of tissues and organs (including the brain and kidneys) did not reveal any treatment-related alterations. Thus, the only treatment-related change was increased water consumption. Similar to the consideration mentioned in the rat study, this change is not considered an adverse effect due to a lack of corresponding effects in kidney pathology and serum electrolyte levels. Therefore, the high dose of 1000 mg/kg-day can be defined as a freestanding NOAEL in this study.

The U.S. EPA (1990a) summarized a lifetime study from the Russian literature (Korolev et al., 1980), but it also indicated that the animal species, the method of administration, and the duration of the study were not reported. Based on this information, this study is not considered in this assessment.

Reproduction/Developmental Studies—Reproductive organs and sex glands have been examined in both rat and mouse studies (Morgan et al., 1988a; Frost et al., 1988). No significant alterations in organ weight or in the gross or microscopic appearance of the reproductive organs from male and female Sprague-Dawley rats and ICR mice treated with up to 1000 mg/kg-day nitroguanidine were reported in the 90-day dietary studies summarized above.

In a developmental study, groups of presumed-pregnant Sprague-Dawley rats (23−27/dose level) were administered 0 (vehicle), 100, 316, or 1000 mg/kg-day nitroguanidine (99.2% pure) in 1% carboxymethylcellulose sodium salt solution by oral gavage on gestation days 6−15 (Coppes et al., 1988a, unpublished report). The dose for each female was based on the body weight at gestation day 6, and that dose was used throughout the treatment period. Maternal gravid body weight and food consumption were monitored on gestation days 0, 6, 10, 15, and 20. Dams were monitored daily for clinical signs of toxicity, abortion, or premature delivery. On gestation day 20, the dams were sacrificed and the gravid uteri were examined for number of implantation sites, resorptions, and live and dead fetuses. The fetuses, uterus, and ovaries were removed; the *corpora lutea* were counted; and the dams were examined for gross visceral signs of toxicity, and the remaining maternal body was weighed. Each fetus was sexed, weighed, measured crown-to-rump, and examined externally. Half of the fetuses were assigned to skeletal examination and the other half to visceral examination.

A total of 7 rats died during the study; five of these deaths were caused by difficulties related to the administration of the test material, and the other two deaths occurred in the high-dose group on gestation days 14 or 16. In addition, 1 dam from the high-dose group was sacrificed due to a moribund condition. Relative to the controls, high-dose dams lost weight (8.5%) during treatment days and showed decreased weight gain (49%) during gestation day 0−20. Food consumption was also significantly reduced in high-dose dams during only the gestation days 6−15 (46%). Clinical signs occurred in 100% of the high-dose group versus 39% of the control group during the treatment period. These clinical signs included red urine, dehydration, red material on the nose/whiskers, red material on the forelimbs, and hunched posture. During the posttreatment period, 29% of the high-dose group showed clinical signs versus 9% of the control group. Treatment with nitroguanidine had no significant effect on the

number of *corpora lutea*, implantations, resorptions, or live and dead fetuses. The high-dose fetuses were significantly lighter and shorter in length than the controls. Significant treatment-related morphological alterations were limited to increased incidences of skeletal variations in the high-dose fetuses, including fewer ossified sternebrae and caudal vertebrae, and reduced ossification of the pubis. Treatment with nitroguanidine did not significantly increase the incidence of external, visceral, or skeletal malformations in treated groups relative to controls. Because the rats treated with the same 90-day dietary dose (1000 mg/kg-day) (Morgan et al., 1988a) did not show increases in mortality rate, the two deaths and the one moribund rat in the high-dose group suggested that the high concentrations of nitroguanidine necessary to administer the 1000 mg/kg-day dose by oral gavage interfered with the digestive processes of the animals in this group. This was supported by other signs of maternal toxicity (e.g., decreased food consumption, decreased body weight gain, and increased incidence of clinical signs) that were present in the same dose group. Based on increased maternal mortality, the alterations in maternal body weight and food consumption, and the clinical signs observed in high-dose dams, the 1000 mg/kg-day dose level can be considered a maternal LOAEL, and the next lower dose of 316 mg/kg-day can be considered a maternal NOAEL. Based on decreased fetal weights and increased incidence of skeletal variations, these dose levels also represent developmental LOAEL and NOAEL values, respectively.

A similar study was conducted with New Zealand White rabbits (Coppes et al., 1988b, unpublished report). Bred females (16−22/dose level) were dosed daily with 0 (vehicle), 100, 316, or 1000 mg/kg-day nitroguanidine (99.2% pure) in 1% carboxymethylcellulose sodium salt solution by oral gavage on day 6 through day 18 of gestation. The dose for each female was based on the gestation day 6 body weight and that dose was used throughout the treatment period. Females were weighed on days 0, 6, 16, 23, and 29; they were observed daily for clinical signs of toxicity, abortion, or premature delivery. Food consumption was calculated daily. Sacrifices were conducted on day 29 of gestation. Non-pregnant rabbits were removed from the study. Gravid uteri were examined for number of implantation sites, resorptions, and live and dead fetuses. The fetuses, uterus, and ovaries were removed; the *corpora lutea* were counted; each dam was examined for gross visceral signs of toxicity; and the remaining maternal body was weighed. Each fetus was weighed, measured crown-to-rump, and examined externally. The viscera from the fetuses were examined for anomalies and the sex was determined. The fetal skeleton was prepared and examined for malformations, alignment, and degree of ossification.

A total of 6 high-dose rabbits died during the study between days 11 and 19, and 4 additional moribund rabbits were euthanized. All these animals had lost significant weight between the first dosing day and the day of death. Food consumption in these animals was significantly reduced. Clinical signs observed in these animals included thick, foamy, granular orange-rust-colored urine, convulsions, hypertonia, and hunched posture. Gross necropsy revealed congestion of the internal organs in most of these rabbits. In the high-dose animals that survived, food consumption during the treatment period was significantly lower than in the controls and weight loss was recorded. Thick, foamy, orange-rust-colored urine and decreased amount of feces occurred frequently in the high-dose groups. Clinical signs occurring only in the high-dose group included hunched posture, hypertonia, increased startle reflex, and death or moribund condition. Nitroguanidine had no significant effect on the number of *corpora lutea*, implantations, live and dead fetuses, or the sex ratio. Fetal weight was significantly reduced in

the high-dose group compared to the control group. Evaluation of visceral and skeletal development showed that the number of fetuses with skeletal variations in the high-dose group was significantly higher than in the control group. The variants most frequently observed were reduced ossification of the sternebrae, olecranon, patella, and phalanges. No treatment-related malformations were observed.

The most significant finding of the study was an increase in the number of gravid females with resorptions at examination on day 29 (3/13 [23%], 13/15 [87%], 7/15 [47%], and 5/11 [45%] in the control, low-, mid-, and high-dose groups, respectively) (Coppes et al., 1988b). According to the investigators, the differences from the controls were statistically significant in the low- and high-dose groups using Marascuilo's method of multiple comparison of proportions. Fisher's Exact Test performed for this review found statistical significance in both low- and mid-dose groups, but it did not show significant response in the high-dose group. In addition, a Cochran-Armitage trend test for these data (gravid females with resorptions) did not demonstrate statistical significance. The investigators also reported that the percent resorptions per litter [(resorptions/implantations) × 100] was significantly higher in treated groups than in controls (2.2%, 9.3%, 6.6%, and 17.9%[1] in the control, low-, mid-, and high-dose groups, respectively). A further review of the number of resorptions by the study authors prompted a letter from the investigators to the U.S. EPA providing additional information [Appendix B in U.S. EPA (1990a)]. In the letter, the investigators stated that the percent resorptions per litter for control rabbits (2.2%) was lower than found in historical control data for New Zealand White rabbits (3.3−16%) from the recent literature (historical data were not available at the study laboratory). The study investigators further stated, "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 (page B-1)." However, the preferential use of literature derived historical control data from other laboratories over the concurrent control data in the interpretation of study findings is in conflict with the principles and practices described in the Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b). Therefore, it is not appropriate to use such background data as a criterion to discount the significance of the observed resorptions in this study. In addition, it is not uncommon to see more sensitive developmental responses in rabbits than in the rats, and there is no evidence suggesting the contrary. The high percent resorptions per litter in the high-dose group were also consistent with the apparent maternal toxicity evidenced by increased mortality and moribund condition and clinical signs. Based on these considerations, it would appear that the low dose of 100 mg/kg-day could be considered a developmental, freestanding LOAEL on the grounds that it caused a statistically significant increase in the percentage of resorptions compared with current control group. This conclusion is in disagreement with the interpretation of this study in U.S. EPA (1990a) and on IRIS (U.S. EPA, 1989). The highest dose level is a maternal frank effect level (FEL) as it caused deaths in the dams; the maternal NOAEL is 316 mg/kg-day.

The reproductive and fertility toxicity of nitroguanidine was assessed in a two-generation study (Coppes et al., 1990, unpublished report). Nitroguanidine (99.7% pure) was fed to Sprague-Dawley rats (parental [F_0] generation) in diet concentrations of 0, 1.3, 4.0, or 12.7 parts

[1] Percent resorption in the high-dose group was reported as 9.7% in the original report (Coppes et al., 1988b). The corrected value of 17.9% was reported by the researchers in a letter to U.S. EPA (see Appendix B of U.S. EPA, 1990a), and it was also confirmed with original data reported in the Appendix I of Coppes et al. (1988b).

per thousand (ppt) starting at 56−58 days of age and continuing throughout mating (24−25 pairs/dose mated after 10 weeks of treatment), gestation, and lactation. Pairs of F_1 offspring (19−26/dose) received the same treated feed as the F_0 animals and were mated when approximately 18-weeks old to produce an F_2 generation, which was evaluated through weaning. In young-adult rats, the consumption of nitroguanidine in the treated feed reportedly approximated the 100, 316, and 1000 mg/kg-day dosages in the developmental studies in rats and rabbits summarized above. Endpoints that were evaluated include clinical observations, food consumption (adult and pup), body weight (adult, pup, and litter), male and female mating and fertility indices, gestation length, numbers of pups delivered, litter size and number, and pup and litter survival indices. Pathological examinations included gross pathology (all dose groups) and histopathology including the reproductive system (control and high-dose groups) in F_0 and F_1 adult rats at end of breeding (males) or end of lactation (females). Gross pathology was evaluated in F_1 and F_2 rats at age 21 days (weaning).

Nitroguanidine had some effect on adult body weight, predominantly in the F_1 generation at 1000 mg/kg-day. Body weights in the 1000 mg/kg-day F_1 males and females were significantly lower than controls (7.6% and 4.9%, respectively) from postweaning week 10 until week 18 or 19 (mating). Maternal gestation body weights were significantly lower than controls in the F_0 high-dose females on gestation days 7, 14, and 21 (7.7, 7.4, and 8.1%, respectively), in the low-dose F_1 females on gestation day 21 (6.6%) and in the high-dose F_1 females on gestation days 14 and 21 (7.8 and 10.5%, respectively). Maternal postpartum body weights were significantly lower than controls in the F_0 high-dose females on lactation days 0 (7.2%) and 14 (8.3%) and in the F_1 high-dose females on lactation days 0 (8.9%), 7 (11.4%), and 14 (8.1%). Food consumption was reduced in both sexes at 1000 mg/kg-day for a few sporadic weeks during the study. The only observed dose-related effect was reduced body weight in the F0 and F1 females during gestation and lactation; however, this effect is not considered adverse because most of these changes were less than 10% compared to the control values. No dose-related toxic effects on reproduction, fertility, or other outcomes were observed. Thus, a NOAEL of 1000 mg/kg-day for reproductive effects and systemic toxicity in adults can be defined in this study.

Inhalation Exposure

No subchronic, chronic, developmental, or reproduction studies on inhaled nitroguanidine in animals are identified.

Other Studies

Acute Studies

The information below has been extracted from U.S. EPA (1990a). In rats, the oral LD_{50} for nitroguanidine in water was 4640 mg/kg and in sunflower-seed oil it was 10,200 mg/kg. In mice and guinea pigs, the oral LD_{50} in sunflower-seed oil was 3850 and 3120 mg/kg, respectively. Other studies reported median lethal doses of >5620 mg/kg and 4340 mg/kg in male and female ICR mice, respectively, administered nitroguanidine in methylcellulose/Tween 80 and a median lethal dose of >5620 mg/kg for male and female Sprague-Dawley rats also treated with nitroguanidine in methylcellulose/Tween 80. Signs of CNS stimulation, such as

seizures, were associated with high doses of nitroguanidine. In general, necropsies did not reveal significant histopathology other than signs of gastric and intestinal irritation. In a 14-day dietary study, doses of 1000 mg/kg-day nitroguanidine increased water consumption and decreased serum calcium and potassium levels in Sprague-Dawley rats (Morgan et al., 1988b). In another study, no significant effects on survival, food intake, weight gain, or gross pathology were noted in male albino rats that received up to 930 mg/kg-day nitroguanidine for 30 days (American Cyanamid, 1955).

Dermal application of a nitroguanidine paste in doses up to 10,000 mg/kg for 24 hours to albino rabbits produced no signs of toxicity or primary skin irritation, and no significant gross pathology was reported. Nitroguanidine was classified as a "nonirritating chemical" in New Zealand White rabbits using the modified Draize method for skin irritation. In another study, application of 500 mg of nitroguanidine to the shaved intact dorsal surface of New Zealand White rabbits for 4 hours did not induce dermal irritation, erythema, or edema during a 14-day observation period. Nitroguanidine was not a dermal sensitizer in a test conducted in Hartley guinea pigs. Direct application of powdered nitroguanidine to the eye on New Zealand White rabbits induced slight conjunctival inflammation 1 and 4 hours post-treatment, but nitroguanidine was not found to be an eye irritant.

Genotoxicity Studies

Assays for the genotoxicity of nitroguanidine (U.S. EPA, 1990a) have generally yielded negative responses, including assays for mutagenicity in *Salmonella typhimurium* and L5178Y mouse lymphoma cells, a mitotic recombination assay in *Saccharomyces cerevisiae*, dominant lethal assays in rats and mice, a sister chromatid exchange assay in Chinese hamster ovary cells (Harbell et al., 1988), a sex-linked recessive lethal mutation assay with *Drosophila melanogaster* (Gupta et al., 1993), and a DNA damage (unscheduled DNA synthesis) assay in human embryonic lung WI-38 cells. However, there was limited evidence of chromosome aberrations in Chinese hamster fibroblast (lung) cells at high doses that may have been cytotoxic (U.S. EPA, 1990a). Overall, the available data suggest that nitroguanidine is not a genotoxic hazard.

DERIVATION OF A PROVISIONAL SUBCHRONIC ORAL p-RfD FOR NITROGUANIDINE

There is an RfD of 0.1 mg/kg-day for nitroguanidine on IRIS (U.S. EPA, 1989). The RfD is based on a LOAEL of 1000 mg/kg-day and a NOAEL of 316 mg/kg-day for reduced weight gain in female rats in a 90-day dietary study (Morgan et al., 1988a), maternal and fetal toxicity in rats exposed during gestation (Coppes et al., 1988a), and evidence of maternal mortality and developmental toxicity in rabbits (Coppes et al., 1988b). However, reanalysis of the rabbit developmental data (Coppes et al., 1988b) in this assessment led to the conclusion that the 100 mg/kg-day dose level was a freestanding LOAEL for developmental effects. Thus, the subchronic oral p-RfD for nitroguanidine was derived using the LOAEL of 100 mg/kg-day as the POD.

Both 90-day studies evaluated clinical signs, hematology, clinical chemistry parameters, and gross and microscopic appearance of tissues and organs. Nitroguanidine exhibited little toxicity in these studies. High-dose female rats gained less weight than controls beginning on week 5 of the study, but the difference between the two groups was less than 10%. Final body weight in the high-dose females was 8.8% lower than controls. This effect may have been related to a significant decrease in food consumption that occurred on weeks 5 and 6. Both studies also showed treatment-related increases in water consumption; however, there was no evidence of kidney pathology or disturbed serum electrolytes in the treated animals. In mice (Frost et al., 1988), the only significant effect from nitroguanidine exposure was an increase in the relative brain weight of male mice at interim sacrifice on day 45 of the study. In contrast to the response in male mice, a similar effect was only observed in female rats (Morgan et al., 1988a), and this effect could have been related to the reduced growth rate in treated rats. Neither study reported positive pathological findings in the brain tissue. Based on the lack of any biologically significant consistent adverse effect, the highest dose level of 1000 mg/kg-day represents a NOAEL in these 90-day studies.

In the rat developmental study, nitroguanidine at 1000 mg/kg-day significantly reduced food consumption and rats lost weight during treatment days (Coppes et al., 1988a). This could have caused, at least in part, the fetuses in this treated group to be significantly lighter in weight and shorter in length than those in the control group. Skeletal variations were also increased at this dose level, and significantly fewer ossified sternebrae and caudal vertebrae were reported. No developmental effects were observed in the 316 mg/kg-day dose group. In pregnant rabbits, exposure to nitroguanidine in dose amounts of 1000 mg/kg-day produced clinical signs and death in the does, reduced food consumption during the treatment period (gestation days 6−18), and weight loss (Coppes et al., 1988b). This dose level significantly reduced fetal weight, but not body length, and increased the incidence of skeletal variations. A significant finding in this study is that treatment with nitroguanidine significantly increased the percentage of resorptions per litter in all treated groups (as low as 100 mg/kg-day), although a dose-response trend was not apparent as shown in a Cochran-Armitage trend test. As mentioned above, a further review of the data revealed that the percent resorptions in the treatment dose as low as 100 mg/kg-day should be considered significantly different from the controls, and as a freestanding LOAEL in this study. The 2-generation reproductive study only identified a high NOAEL of 1000 mg/kg-day.

The lowest LOAEL in the overall database is 100 mg/kg-day for developmental effects (increased resorptions) in rabbits. Although all the treated groups in the rabbit study showed significantly increased resorptions, there is significant noise in the dose response relationship in the data. For the litter-based response (percentage of gravid females with resorptions), the lowest dose group presented a far greater response (87%) than those in the mid- and high-dose groups (45−47%). Similarly, the pup-based resorption rate in the low-dose group (9.3%) was higher than that in the mid-dose group (6.6%). Thus, these data are not amenable to BMD modeling. Therefore, the LOAEL of 100 mg/kg-day is used as the POD for the derivation of the subchronic p-RfD.

The **subchronic p-RfD of 0.1 mg/kg-day** is calculated by applying a composite uncertainty factor of 1000 to the developmental POD of 100 mg/kg-day, as follows:

Subchronic p-RfD = LOAEL ÷ UF
= 100 mg/kg-day ÷ 1000
= **0.1 mg/kg-day or 1×10^{-1} mg/kg-day**

The composite UF of 1000 was composed of the following:

- An UF of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between laboratory animals and humans.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF of 10 is applied because a LOAEL, instead of NOAEL, was used as the POD.
- An UF is not applied to account for uncertainty in the database. The database includes comprehensive subchronic studies in 2 animal species, 2 developmental toxicity studies in 2-animal species and a 2-generation reproduction study in rats.

Confidence in the critical study is medium. It was a well conducted GLP study that had an adequate number of animals and evaluated comprehensive toxicological endpoints. However, the study did not identify a NOAEL, and the dose-response data for the critical effect (i.e., increased resorptions rate) may be confounded by high mortality and moribund rate in the high-dose group. Confidence in the database is high. The available database is comprehensive, including 2 subchronic studies in 2 animal species, 2 developmental studies in 2 species, and a 2-generation reproductive study. In considering the confidences in both the key study and the database, the overall confidence in the resulted subchronic p-RfD is medium.

The chronic RfD of 0.1 mg/kg-day is currently listed on IRIS (U.S. EPA, 1989); therefore, no chronic p-RfD is derived in this document. Note that the IRIS RfD is based on systemic toxicity from several studies (reduced weight gain in female rats, developmental and maternal toxicity at 1000 mg/kg-day body weight). This subchronic p-RfD is based on reproductive effects at 100 mg/kg-day in one of the same studies.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION p-RfC VALUES FOR NITROGUANIDINE

No studies investigating the effects of subchronic or chronic inhalation exposures to nitroguanidine in humans or animals are identified. The lack of suitable data precludes derivation of subchronic and chronic p-RfCs for nitroguanidine.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR NITROGUANIDINE

Weight-of-Evidence Descriptor

Studies evaluating the carcinogenic potential of oral or inhalation exposure to nitroguanidine in humans or animals were not identified in the available literature. The available genotoxicity data suggest that nitroguanidine is not a genotoxic hazard. Nitroguanidine is classified on IRIS (U.S. EPA, 1990b) in Group D, *not classifiable as a human carcinogen* on the basis that pertinent data regarding carcinogenicity were not located in the available literature. Under the 2005 Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 2005), nitroguanidine is characterized as "*Inadequate Information to Assess the Carcinogenic Potential*."

Quantitative Estimates of Carcinogenic Risk

The lack of suitable data precludes the derivation of quantitative cancer risk estimates for nitroguanidine.

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