Nitrate; CASRN 14797-55-8

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Nitrate

File First On-Line 01/31/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	05/01/1991
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	not evaluated	

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Nitrate
CASRN —14797-55-8
Last Revised — 05/01/1991

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Early clinical signs of methemoglobinemia in excess of 10% (0-3 months old infants formula) **Human Epidemiological Surveys** **Bosch et al., 1950; Walton, 1951**	NOAEL: 10 mg nitrate-nitrogen/L (1.6 mg/kg/day) LOAEL: 11-20 mg nitrate-nitrogen/L (1.8-3.2 mg/kg/day)	1	1	1.6E+0 mg/kg/day

*Conversion Factor: Expressed as the amount of nitrogen within the nitrite molecule commonly shown as mg nitrate-nitrogen/L (1 mg nitrate-nitrogen = 4.4 mg nitrate). Doses based on ingestion of drinking water used to prepare infants' formula: 0.64 L/day by a 4 kg infant (0.16 L/kg/day) (Davidson et al., 1975). 10 mg/L x 0.64 L/day divided by 4 kg = 1.6 mg/kg/day.

I.A.2. Principal and Supporting Studies (Oral RfD)

Bosch, H.M., A.B. Rosefield, R. Huston, H.R. Shipman and F.L. Woodward. 1950. Methemoglobinemia and Minnesota well supplies. J. Am. Water Works Assoc. 42: 161-170.

Walton, G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am. J. Public Health. 41: 986-996.

Most cases of infant methemoglobinemia are associated with exposure to nitrate in drinking water used to prepare infants' formula at levels >20 mg/L of nitrate-nitrogen (Bosch et al., 1950; Walton, 1951; Sattelmacher, 1962; Simon et al., 1964; ECETOC, 1988). Cases reported at levels of 11-20 mg/L nitrate- nitrogen are usually associated with concomitant exposure to bacteriologically contaminated water or excess intake of nitrate from other sources.

Bosch et al. (1950) evaluated 139 cases of cyanosis due to methemoglobinemia reported by physicians in Minnesota. All of the cases were in young children (ages 8 days to 5 months), with 90% occurring in infants <2 months of age. A study of the nitrate concentration of the wells (a total of 129) used to supply water to the children with methemoglobinemia was performed. None of the wells contained <10 mg/L nitrate-nitrogen. Two wells (1.5%) contained 10- 20 mg/L, although the diagnosis of methemoglobinemia was considered questionable in both these cases. There were 25 wells (19%) that contained 21-50 mg/L, 53 (41%) that contained 51-100 mg/L, and 49 (38%) that contained >100 mg/L nitrate-nitrogen. Nearly all the wells were shallow with inadequate protection from surface contamination. Coliform organisms were detected in 45 of 51 samples (88%) tested for bacterial contamination.

Walton (1951) described a survey performed by the American Public Health Association to identify clinical cases of infantile methemoglobinemia that were associated with ingestion of nitrate-contaminated water. A total of 278 cases of methemoglobinemia were reported. Of 214 cases for which data were available on nitrate levels in water, none occurred in infants consuming water containing <10 mg nitrate-nitrogen/L (1.6 mg nitrate-nitrogen/kg/day). There were 5 cases (2%) in infants exposed to 11-20 mg nitrate-nitrogen/L (1.8-3.2 mg/kg/day), 36 cases (17%) in infants exposed to 21-50 mg/L (3.4-8.0 mg/kg/day), and 173 (81%) in infants exposed to >50 mg/L (>8 mg/kg/day). Data on the ages of the infants were not provided.

Cornblath and Hartmann (1948) supplied nitrate-containing water to eight healthy infants (ages 2 days to 11 months) at doses of 50 or 100 mg NO3/kg/day (11 or 23 mg nitrate-nitrogen/kg/day). Assuming average consumption of about 0.16 L/kg/day, this corresponds to concentrations of 70 or 140 mg nitrate- nitrogen/L. No cyanosis was evident in any infant, and the highest concentration of methemoglobin was 7.5%. These authors also administered doses of 100 mg/kg of nitrate to four healthy infants (age 2 days to 6 months) and to two infants (age 6 and 7 weeks) who had been admitted to the hospital for cyanosis. No cyanosis was produced in the healthy infants, but cyanosis did occur in the individuals with a prior history of cyanosis. Examination of the saliva, gastric juice and stools of these infants revealed the presence of bacteria that readily reduced nitrate to nitrite. The gastric pH of these infants was >4 in both cases.

Donahoe (1949) reported five cases of moderate to severe cyanosis in infants (age 1-7 weeks) in South Dakota. In four of the five cases, the water used to feed the infants was from shallow wells and was shown to be heavily contaminated with bacteria. Nitrate levels were measured in two cases, with values of 50 and 177 mg/L (12 and 41 mg nitrate-nitrogen/L), respectively. This corresponds to doses of 8 and 28 mg nitrate-nitrogen/kg/day.

Simon et al. (1964) measured methemoglobin levels in 89 healthy infants who received nitrate-free water, 38 infants who received water containing 11-23 mg nitrate-nitrogen/L (1.8-3.7 mg nitrate-nitrogen/kg/day), and 25 infants receiving water containing >23 mg nitrate-nitrogen/L

(>3.7 mg nitrate- nitrogen/kg/day). For infants age 1-3 months, mean methemoglobin levels in these three groups were 1.0, 1.3 and 2.9%, respectively. For infants age 3-6 months, values were 0.8, 0.8 and 0.7%, respectively. No clinical signs of methemoglobinemia were detected in any of the infants.

Toussaint and Selenka (1970) supplied 34 healthy infants (age 1-3 months) with formula prepared with water containing 150 mg nitrate/L (34.5 mg nitrate- nitrogen/L, corresponding to 5.5 mg nitrate-nitrogen/kg/day). Average methemoglobin levels rose from about 1% to about 2-3% within 1-2 days, and then tended to stay steady for up to 10 days. No clinical signs of methemoglobinemia were reported.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 1 was employed because available data define the no-observed-adverse-effect level for the critical toxic effect in the most sensitive human subpopulation.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

Nitrate toxicity is due primarily to its conversion to nitrite, which oxidizes the Fe(+2) form of iron in hemoglobin to the Fe(+3) state. This compound (methemoglobin) does not bind oxygen, resulting in reduced oxygen transport from lungs to tissues. Low levels of methemoglobin occur in normal individuals, with typical values usually ranging from 0.5 to 2.0% (NAS, 1981). However, due to the large excess capacity of blood to carry oxygen, levels of methemoglobin up to around 10% are not associated with any significant clinical signs (Walton, 1951; ECETOC, 1988). Concentrations above 10% may cause a bluish color to skin and lips (cyanosis), while values above 25% lead to weakness, rapid pulse and tachypnea (Jones et al., 1973). Death may occur if methemoglobin values exceed 50-60%.

Conversion of nitrate to nitrite is mostly mediated by bacteria in the gastrointestinal system. Consequently, the risk of methemoglobinemia from ingestion of nitrate depends not only on the dose of nitrate, but also on the number and type of enteric bacteria. In healthy adults, available data suggest about 5% of a dose of nitrate is reduced to nitrite by bacteria in the mouth (NAS, 1981). Conversion of nitrate to nitrite may also occur in the stomach if the pH of the gastric fluid is sufficiently high (above pH 5) to permit bacterial growth. This is of concern in adults with diseases such as achlorhydria or atrophic gastritis. It is also of concern in infants, since the infant gastrointestinal system normally has a high pH that favors the growth of nitrate-reducing bacteria. For this reason, infants (especially age 0-3 months) are generally recognized as being the subpopulation most susceptible to nitrate-induced methemoglobinemia. Risk is especially

high in infants who are exposed to water that is contaminated with bacteria, since this tends to promote high concentrations of bacteria in the stomach and intestines.

Nitrate is a normal component of the human diet. A typical daily intake by an adult in the United States is about 75 mg/day (about 0.2-0.3 mg nitrate- nitrogen/kg/day) (NAS, 1981). Of this, over 85% comes from the natural nitrate content of vegetables such as beets, celery, lettuce and spinach. Daily intakes of nitrate by vegetarians may exceed 250 mg/day (0.8 mg nitrate-nitrogen/kg/day) (NAS, 1981). The contribution from drinking water is usually quite small (about 2-3% of the total) (NAS 1981), but could reach 85 mg/day (0.29 mg nitrate-nitrogen/kg/day) if water containing 10 mg nitrate-nitrogen/L was consumed. Thus, some adults consuming high levels of vegetables along with water containing high levels of nitrate (up to 10 mg nitrate-nitrogen/L) could receive total doses of nitrate approaching the RfD of 1.6 mg nitrate-nitrogen/kg/day.

Two epidemiological studies have been performed on the adverse effects of nitrate exposure, but the results are internally inconsistent or inconclusive. Dorsch et al. (1984) found a statistically significant increase in risk of birth defects in children of women consuming groundwater (which contained 5-15 mg/L of nitrate) compared with women consuming rainwater (which contained <5 mg/L nitrate). These authors emphasized that their results are limited by a number of factors, and stated that "it would be premature to interpret our case-control findings exclusively in terms of water nitrate exposure." Arbuckle et al. (1988) reported nonstatistically significant increase in the odds ratio for birth defects in children of women exposed to well-water (26 mg/L nitrate, equivalent to 0.2 mg nitrate-nitrogen/kg/day) compared with rain water (0.1 mg/L nitrate, equivalent to 0.0008 mg nitrate-nitrogen/kg/day). However, decreased odds ratios (also not statistically significant) were noted for exposure to nitrate in spring water (17 mg/L, equivalent to 0.13 mg nitrate-nitrogen/kg/day) or public water (26 mg/L).

Craun et al. (1981) conducted an epidemiologic study of 102 children aged 1-8 years in Washington County, Illinois. Sixty-four children were selected from families consuming high-nitrate water (22-111 mg/L nitrate-nitrogen) and 38 children (controls) were from families consuming water containing <10 mg/L nitrate-nitrogen. Ingestion of high-nitrate water was not found to result in above-normal methemoglobin levels in exposed children. Assuming ingestion of 0.1 L/kg/day by older children, these concentrations correspond to doses of 2.2-11 mg nitrate-nitrogen/kg/day. This study indicates that older children are much less susceptible to nitrate-induced methemoglobinemia than are infants.

The Food and Drug Administration sponsored extensive tests of the reproductive and developmental effects of $NaNO_3$ and KNO_3 in mice, rats, hamsters and rabbits (FDA, 1972a,b). Groups of 20-26 mice, rats or hamsters and 10-13 rabbits were treated by gavage on days 6-15 (mice, rats), days 6-10 (hamster) or days 6-18 (rabbits) of gestation. Fetuses were delivered by

Cesarean section and examined for visceral and skeletal malformations. Dose levels (expressed as mg nitrate-nitrogen) ranged from 0.6-66 mg/kg/day for mice, from 0.3-41 mg/kg/day for rats, from 0.4-66 mg/kg/day for hamsters and from 0.3-41 mg/kg/day for rabbits. No significant effects were detected regarding maternal reproductive parameters (percent pregnant, abortion frequency, number of litters), fetotoxicity (percent fetal resportions, live fetuses per dam, average fetal weight) or fetal malformations up to the maximum doses administered to each species. These studies identify a reproductive/developmental NOAEL of 66 mg nitrate-nitrogen/kg/day for mice and hamsters and 41 mg nitrate-nitrogen/kg/day for rats and rabbits.

Sleight and Atallah (1968) studied the effects of nitrate on reproduction and development in guinea pigs. Groups of 3-6 females were exposed to drinking water containing 0, 300, 2500, 10,000 or 30,000 ppm KNO3 for 143-204 days. This resulted in average doses of 0, 12, 102, 507 or 1130 mg nitrate- nitrogen/kg/day. Normal conception occurred at all dose levels. No significant effect on reproductive performance was detected except in the high-dose group, where there was a decrease in number of live births. The authors attributed the fetotoxic effects to hypoxia due to maternal methemoglobinemia, although data on this were not provided. No fetal malformations were observed at any dose. This study identifies a reproductive NOAEL of 507 and a LOAEL of 1130 mg nitrate-nitrogen/kg/day.

No multi-generation studies were located on the reproductive effects of nitrate. In the absence of such data, observations from animals exposed to nitrite may be used as a conservative estimate of nitrate toxicity.

Hugot et al. (1980) performed a three-generation study in rats. Female animals were administered sodium nitrite in the diet at doses of 90 or 160 mg nitrite-nitrogen/kg/day. There were no effects on a number of reproductive parameters. Some pups showed small decreases in birth weight and growth rate during lactation, and changes in organ weights at weaning. This study identifies a LOAEL of 90 mg nitrite-nitrogen/kg/day. Assuming that a maximum of 10% of a dose of nitrate is converted to nitrite by an adult human, this would correspond to a LOAEL of 900 mg nitrate-nitrogen/kg/day.

Druckrey et al. (1963) supplied rats with NaNO2 in drinking water for three generations at a dose level of 100 mg/kg/day (20 mg nitrite-nitrogen/kg/day). No teratogenic effects or adverse effects on reproduction were detected in any generation. Assuming that a maximum of 10% of a dose of nitrate is converted to nitrite by an adult human, this would correspond to a NOAEL of 200 mg nitrate-nitrogen/kg/day.

No studies were located on systemic effects of nitrate in humans or animals. In the absence of such data, observations from animals exposed to nitrite may be used as a conservative estimate of nitrate toxicity. Druckrey et al. (1963) exposed rats for their lifetime to NaNO2 in drinking

water at a dose of 100 mg/kg/day (20 mg nitrite-nitrogen/kg/day). No treatment-related histologic or hematologic effects were noted except for elevated methemoglobin levels in the treated animals.

Til et al. (1988) supplied rats with drinking water containing up to 3000 mg/L of KNO2 (500 mg nitrite-nitrogen/L, equivalent to 50 mg nitrite- nitrogen/kg/day) for 13 weeks. No histological effects were detected except for a very slight to slight hypertrophy of the zona glomerulosa. This was probably due to reduced water intake, and is not judged to constitute an adverse health effect. This study identifies a NOAEL of 17 and a LOAEL of 50 mg nitrite-nitrogen/kg/day (based on methemoglobin levels). Assuming that a maximum of 10% of a dose of nitrate is converted to nitrite by an adult human, this would correspond to a NOAEL of 170 and a LOAEL of 500 mg nitrate- nitrogen/kg/day.

Shuval and Gruener (1972) exposed rats for 24 months to water containing 0, 100, 1000, 2000 or 3000 ppm of sodium nitrite (0, 2, 20, 40 or 60 mg nitrite- nitrogen/kg/day). Histological examination of the lungs revealed dilated bronchi, fibrosis and emphysema at 1000 ppm or above. Histological examination of the heart revealed an increased percentage of coronary arteries that were characterized as "thin and dilated." This effect appears to be at least partly due to the absence of coronary artery thickening and narrowing that normally occurs in aged rats, so it is not certain that these changes are inherently adverse. Based on effects on the lung, this study identifies a NOAEL of 2 and a LOAEL of 20 mg nitrite-nitrogen/kg/day. Assuming that a maximum of 10% of a dose of nitrate is converted to nitrite by an adult human, this would correspond to a NOAEL of 20 and a LOAEL of 200 mg nitrate- nitrogen/kg/day.

I.A.5. Confidence in the Oral RfD

Study — High
Database — High
RfD — High

The studies of Bosch et al. (1950) and Walton (1951) provide convincing evidence that infantile methemoglobinemia does not occur at drinking water levels of 10 mg nitrate-nitrogen/L or less. This is supported by a large number of additional epidemiological and case studies in humans (e.g., Cornblath and Hartmann, 1948; Simon et al., 1964; Toussaint and Selenka, 1970; Craun et al., 1981; see U.S. EPA, 1990 for descriptions of additional studies).

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — This assessment is not presented in any existing U.S. EPA document.

Other EPA Documentation — U.S. EPA, 1990

Agency Work Group Review — 11/21/1985, 02/05/1986, 02/26/1986, 06/20/1990, 07/25/1990, 08/22/1990

Verification Date — 08/22/1990

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Nitrate conducted in September 2002 identified one or more significant new studies. IRIS users may request the references for those studies from the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Nitrate
CASRN —14797-55-8

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Nitrate
CASRN —14797-55-8

Not available at this time.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — Nitrate
CASRN —14797-55-8

VI.A. Oral RfD References

Arbuckle, T.E., G.J. Sherman, P.N. Corey, D. Walters and B. Lo. 1988. Water nitrates and CNS birth defects: A population-based case-control study. Arch. Environ. Health. 43(2): 162-167.

Bosch, H.M., A.B. Rosefield, R. Huston, H.R. Shipman and F.L. Woodward. 1950. Methemoglobinemia and Minnesota well supplies. J. Am. Water Works Assoc. 42: 161-170.

Cornblath, M. and A.F. Hartmann. 1948. Methemoglobinemia in young infants. J. Pediatr. 33: 421-425.

Craun, G.F., D.G. Greathouse and D.H. Gunderson. 1981. Methaemoglobin levels in young children consuming high nitrate well water in the United States. Int. J. Epidemiol. 10(4): 309-317.

Davidson, S., R. Passmore, J.F. Brock and A.S. Truswell. 1975. Human Nutrition and Dietetics, 6th ed. Churchill Livingstone, New York. p. 644- 645.

Donahoe, W.E. 1949. Cyanosis in infants with nitrates in drinking water as cause. Pediatrics. 3: 308-311.

Dorsch, M.M., R.K.R. Scragg, A.J. McMichael, P.A. Baghurst and K.F. Dyer. 1984. Congenital malformations and maternal drinking water supply in rural South Australia: A case-control study. J. Epidemiol. 119(4): 473-486.

Druckrey, H., D. Steinhoff, H. Beuthner, H. Schneider and P. Klarner. 1963. Screening of nitrite for chronic toxicity in rats. Arzneim. Forsch. 13: 320- 323. (Ger. with Eng. summary)

ECETOC (European Chemical Industry Ecology and Toxicology Center). 1988. Nitrate and drinking water. Technical Report No. 27, Nitrate and Drinking Water. Brussels, Belgium.

FDA (Food and Drug Administration). 1972a. Teratologic evaluation of FDA 71- 7 (sodium nitrate). Food and Drug Administration, Washington, DC. PB 221775.

FDA (Food and Drug Administration). 1972b. Teratologic evaluation of FDA 71-8 (potassium nitrate). Food and Drug Administration, Washington, DC. PB 221774.

Hugot, D., J. Causeret and C. Richir. 1980. The influence of large amounts of sodium nitrite on the reproductive performances in female rats. Ann. Nutr. Alim. 34: 1115-1124.

Jones, J.H., H.T. Sethney, G.W. Schoenhals, R.N. Grantham and H.D. Riley, Jr. 1973. Grandmother's poisoned well: Report of a case of methemoglobinemia in an infant in Oklahoma. Okla. State Med. Assoc. J. 66: 60-66.

NAS (National Academy of Sciences). 1981. The health effects of nitrate, nitrite and N-nitroso compounds. National Academy Press, Washington, DC.

Sattelmacher, P.G. 1962. Methamoglobinamie durch Nitrate im Trinkwasser. Schriftenreie Verein Wasser Boden Lufthyg. Berlin-Dahlem, no. 20. Gustav Fischer Verlag, Stuttgart. (Ger.)

Shuval, H.I. and N. Gruener. 1972. Epidemiological and toxicological aspects of nitrates and nitrites in the environment. Am. J. Public Health. 62(8): 1045-1052.

Simon, C., H. Manzke, H. Kay and G. Mrowetz. 1964. Occurrence, pathogenesis, and possible prophylaxis of nitrite induced methemoglobinemia. Zeitschr. Kinderheilk. 91: 124-138. (Ger.)

Sleight, S.D. and O.A. Atallah. 1968. Reproduction in the guinea pig as affected by chronic administration of potassium nitrate and potassium nitrite. Toxicol. Appl. Pharmacol. 12: 179-185.

Til, H.P., H.E. Falke, C.F. Kuper and M.I. Willems. 1988. Evaluation of the oral toxicity of potassium nitrite in a 13-week drinking-water study in rats. Food Chem. Toxicol. 26(10): 851-859.

Toussaint, W. and F. Selenka. 1970. Methemoglobin formation in infants. A contribution to drinking water hygiene in Rhine-Hesse. Mschr. Kinderheilk. June: 282-284.

U.S. EPA. 1990. Criteria Document for Nitrate/Nitrite. Office of Drinking Water, Washington, DC.

Walton, G. 1951. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. Am. J. Public Health. 41: 986-996.

VI.B. Inhalation RfC References

None

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — Nitrate
CASRN —14797-55-8

Date	Section	Description
09/01/1990	I.A.	Withdrawn; new Oral RfD verified (in preparation)
05/01/1991	I.A.	Oral RfD summary replaced (RfD changed)