ATSDR Case Studies in Environmental Medicine

Nitrate/Nitrite Toxicity

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

226072-A

Agency for Toxic Substances and Disease Registry

Case Studies in Environmental Medicine (CSEM)

Nitrate / Nitrite Toxicity

Course: WB2342

CE Original Date: December 5, 2013
CE Renewal Date: December 5, 2015
CE Expiration Date: December 5, 2017

Key Concepts	• Nitrate toxicity is a preventable cause of methemoglobinemia. • Infants younger than 4 months of age are at particular risk of nitrate toxicity from contaminated well water. • The widespread use of nitrate fertilizers increases the risk of well-water contamination in rural areas.
About This and Other Case Studies in Environmental Medicine	This educational case study document is one in a series of self-instructional modules designed to increase the primary care provider's knowledge of hazardous substances in the environment and to promote the adoption of medical practices that aid in the evaluation and care of potentially exposed patients. The complete series of Case Studies in Environmental Medicine is located on the ATSDR Web site at URL: http://www.atsdr.cdc.gov/csem/csem.html In addition, the downloadable PDF version of this educational series and other environmental medicine materials provides content in an electronic, printable format.

Acknowledgements	We gratefully acknowledge the work of the medical writers, editors, and reviewers in producing this educational resource. Contributors to this version of the Case Study in Environmental Medicine are listed below. Please Note: Each content expert for this case study has indicated that there is no conflict of interest that would bias the case study content. **CDC/ATSDR Author(s):** Kim Gehle MD, MPH **CDC/ATSDR Planners:** Charlton Coles, Ph.D.; Kimberly Gehle, MD; Sharon L. Hall, Ph.D.; Delene Roberts, MSMHC; Julia Smith, MPH, CHES **CDC/ATSDR Independent Reviewers:** Hana R. Pohl, MD, PhD; Michelle Watters, MD, PhD, MPH **CDC/ATSDR Commenter's:** Art Chang MD, MS; Lorraine Backer PhD, MPH
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U.S. Department of Health and Human Services

Agency for Toxic Substances and Disease Registry

Division of Toxicology and Human Health Sciences

Environmental Medicine Branch

Table of Contents

How to Use This Course

Introduction

The goal of *Case Studies in Environmental Medicine* (CSEM) is to increase the primary care provider's knowledge of hazardous substances in the environment and to assist in the evaluation and treatment of potentially exposed patients. This CSEM focuses on nitrites/nitrates toxicity.

Availability

Two versions of the Nitrites/Nitrates Toxicity CSEM are available.

- The HTML version http://www.atsdr.cdc.gov/csem/csem.asp?csem=28&po=0 provides content through the Internet
- The downloadable PDF version http://www.atsdr.cdc.gov/csem/nitrate_2013/docs/nitrite.pdf provides content in an electronic, printable format.
- The HTML version offers interactive exercises and prescriptive feedback to the user.

Instructions

To make the most effective use of this course.

- Take the Initial Check to assess your current knowledge about nitrites/nitrates toxicity.
- Read the title, learning objectives, text, and key points in each section.
- Complete the progress check exercises at the end of each section and check your answers.
- Complete and submit your assessment and posttest response online if you wish to obtain continuing education credit. Continuing education certificates can be printed immediately upon completion.

Instructional Format

This course is designed to help you learn efficiently. Topics are clearly labeled so that you can skip sections or quickly scan sections with which you are already familiar. This labeling will also allow you to use this training material as a handy reference. To help you identify and absorb important content quickly, each section is structured as follows.

Section Element	Purpose
Title	Serves as a "focus question" that you should be able to answer after completing the section.
Learning Objectives	Describes specific content addressed in each section and focuses your attention on important points.
Text	Provides the information you need to answer the focus question(s) and achieve the learning objectives.
Key Points	Highlights important issues and helps you review.
Progress Check Exercises	Enables you to test yourself to determine whether you have mastered the learning objectives.
Progress Check Answers	Provide feedback to ensure you understand the content and can locate information in the text.

Learning Objectives

Upon completion of the Nitrate/Nitrite CSEM, you will be able to

Overview	• Describe what nitrates and nitrites are.
Exposure Pathways	• Identify sources of nitrates and nitrites. • Describe the primary routes of exposure to nitrates and nitrites.
Who Is at Most Risk of Adverse Health Effects From Overexposure	• Identify the population most susceptible to the adverse health effects from overexposure to nitrates and nitrites.
Standards and Regulations	• Describe the U.S. Environmental Protection Agency's (EPA's) recommended limit for nitrates and nitrites in drinking water. • Describe the U.S. Food and Drug Administration's (FDA's) recommended limit for nitrates and nitrites in bottled water and foodstuffs.
Biological Fate	• Describe what happens to nitrates and nitrites once they enter the body.
Health Effects	• Describe mechanisms contributing to health effects from exposure to nitrates and nitrites. • Describe the health effects from exposure to nitrates and nitrites.
Clinical Evaluation	• Describe the clinical assessment of an infant with cyanosis due to overexposure to nitrates and nitrites. • Describe the signs and symptoms of methemoglobinemia. • Identify the laboratory test results that indicate methemoglobinemia.
Treatment and Management	• Describe treatment modalities in the management of methemoglobinemia caused by nitrate and nitrite toxicity.
Instructions to Patients	• Describe care advice the clinician can provide to patients to prevent overexposure to nitrates and nitrites.

Initial Check

Instructions

This Initial Check will help you assess your current knowledge about nitrate/nitrite toxicity. To take the initial check, read the case below, and then answer the questions that follow.

Case Study

A 2-month-old infant has vomiting, diarrhea, tachypnea, and cyanosis.

A two-month-old female infant is brought to your clinic in a rural area for a routine well-baby checkup. According to the child's chart, she was delivered 2 weeks early because of maternal pre-eclampsia. There was no neonatal distress; her birth weight was 7 pounds and 2 ounces.

Today, the mother states that she has noticed an intermittent bluish discoloration of the baby's:

- Lips,
- Tip of the nose, and
- Ears.

Physical examination of the infant is negative for cardiac murmurs and abnormalities on lung auscultation. You note a below-average weight gain. Feedings consist of 4 ounces of diluted formula every 2 hours. The infant has occasional loose stools. You instruct the parents to increase caloric feedings, which should include vitamin and mineral supplements. You tell the parents to call you immediately if any further episodes of the bluish discoloration occur.

Approximately 3 weeks later, the baby's frantic parents call your office; the infant is crying incessantly and has vomiting and profuse diarrhea.

Vital Signs

When the baby is brought to your clinic a few minutes later, she is afebrile but has tachypnea, central cyanosis, and drowsiness. You note her vital signs as

- Blood pressure (BP) = 78/30 millimeter (mm) mercury (Hg) (normal 50th percentile for her age is 80/46 mm Hg)
- Heart rate = 160 beats/minute (normal range for 0-3 months = 100-150 beats/minute)
- Respiration = 60 breaths/minute (normal range for 0-3 months = 35-55 breaths/minute)

Additional Information

An ambulance is summoned and 100% oxygen is administered by face mask. No improvement in the cyanosis is noted on her arrival at the hospital emergency department.

Emergency Treatment

The examining emergency physician now notes a grade II/VI systolic murmur and central cyanosis, which has not improved despite administration of 100% oxygen for nearly 1 hour. The infant shows no evidence of

- Cardiac failure,
- Atelectasis,
- Pneumonitis, or
- Pneumothorax.

Therapy with methylene blue is started, which results in a dramatic resolution of the cyanosis. The infant is discharged on the second hospital day with no evidence of central nervous system hypoxic damage.

Initial Check

1. Considering the differential diagnosis for cyanosis, what is the most likely cause of this infant's cyanosis?
2. What laboratory tests, either obtained during the hospitalization or ordered subsequently, would help confirm the diagnosis?
3. What steps, if any, can be taken to prevent a recurrence of cyanosis and distress in this infant?
4. What questions will you ask the parents of the infant to help determine the cause of the cyanosis?
5. If well water used to dilute formula is implicated in the cyanosis, what are some possible causes of its nitrate contamination?

6. What recommendations can you make to the infant's family to prevent further cyanotic episodes?
7. What factors make infants younger than 4 months of age more susceptible to developing methemoglobinemia when exposed to nitrates?
8. Why might some patients with methemoglobinemia not respond to treatment with methylene blue?
9. What options are available to treat significant methemoglobinemia in a patient who has glucose 6-phosphate dehydrogenase (G6PD) deficiency?

Initial Check Answers

1. The differential diagnosis for cyanosis in an infant includes (by mechanism)

- Alveolar Hypoventilation
    - CNS depression (i.e., asphyxia, seizure, meningitis, encephalitis, intraventricular hemorrhage, drug induced)
    - Airway obstruction (i.e., choanal atresia, laryngomalacia)
    - Neuromuscular disease (i.e., phrenic nerve injury, myasthenia gravis)
- Decreased oxygen carrying capacity of blood (less oxygen available at tissue level)
    - Methemoglobinemia (acquired or congenital)
- Decreased peripheral circulation (peripheral cyanosis)
    - Sepsis
    - Shock (any cause)
    - Polycythemia
    - Hypothermia
    - Hypoglycemia
    - Low cardiac output (i.e. hypocalcemia, cardiomyopathies)
- Impaired Oxygen Diffusion
    - Pulmonary edema (i.e. left sided obstructive cardiac disease as seen with aortic stenosis, cardiomyopathy)
    - Pulmonary fibrosis
- Right–to–left shunt
    - Cardiac anomalies (i.e. tetralogy of fallot, transposition of the great arteries, truncus arteriosus, total anomalous pulmonary venous return, tricuspid atresia, pulmonary atresia, hypoplastic left heart)
    - Persistent pulmonary hypertension of the newborn

- Pulmonary anomalies (i.e., pulmonary arteriovenous malformation)

- Ventilation/perfusion mismatch

  - Airway disease (i.e. transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), pneumonia, aspiration, atelectasis, diaphragmatic hernia, pulmonary hypoplasia, pulmonary hemorrhage)
  - Extrinsic compression of the lungs (i.e., pneumothorax, pleural effusion, hemothorax)

In an infant with no known cardiopulmonary disease, cyanosis that is unresponsive to oxygen therapy is most likely due to methemoglobinemia.

Methemoglobinemia is a condition of excess oxidized “ferric” hemoglobin where the reducing systems to return hemoglobin to a ferrous state are overwhelmed, impaired, or lacking. Causes of methemoglobinemia can be generally grouped into three categories: endogenous (i.e., related to diarrhea, systemic acidosis, infection); exogenous (i.e., medication or toxin-induced); and genetic (i.e., related to methemoglobin reductase enzyme system deficiency or structural variant of hemoglobin (HbM)).

A high index of suspicion is the key to proper and timely diagnosis. Note that methemoglobinemia can also occur with subtle or no symptoms depending on the methemoglobin level.

Other forms of abnormal hemoglobin (dyshemoglobin) that also have an impaired ability to transport oxygen and carbon dioxide include carboxyhemoglobin and sulfhemoglobin. Therefore, carboxyhemoglobinemia and sulfhemoglobinemia should also be considered in the differential diagnosis.

More detailed information regarding this answer including further discussion of differential diagnosis and clinical work up can be found in

the "What Are Health Effects from Exposure to Nitrate and Nitrites?" and "How Should Patients Potentially Overexposed to Nitrates and Nitrites Be Evaluated (Clinical Assessment)?" sections.

2. Laboratory tests useful for screening a patient with suspected methemoglobinemia include

   - Examination of blood color with bedside "filter paper" (chocolate brown color of blood remains unchanged with exposure to oxygen)
   - Arterial blood gases (ABGs) with co-oximetry (to determine MetHb level, oxygen saturation, presence of other dyshemoglobins, etc.)
   - Complete blood counts (CBC) with peripheral blood smear (can be used to identify and characterize anemias, differentiate hemoglobinopathies from thalassemias, etc.)
   - Serum-free hemoglobin (can be used to detect hemolytic anemias)
   - Serum haptoglobin (can be used to detect hemolytic anemias; i.e., decreased haptoglobin, RBC count, hemoglobin and hematocrit with increased reticulocyte count are supportive of a hemolytic anemia diagnosis)

   More detailed information regarding this answer including further discussion of differential diagnoses and clinical work up can be found in the "How Should Patients Potentially Overexposed to Nitrates and Nitrites Be Evaluated (Clinical Assessment)?" section.

3. The initial step in preventing a recurrence of the infant's cyanosis and distress is to identify the cause of the cyanosis. The next step is to correct or eliminate the cause.

   If the infant's cyanosis is due to an acquired methemoglobinemia, the agent must be identified and removed from the infant's environment. For example, there have been cases of infantile acquired methemoglobinemia through ingestion of baby formula prepared using nitrate contaminated well water. Ingestion of nitrate-containing water is

a common cause of methemoglobinemia in infants, especially those living in rural areas. EPA suggests maintenance testing of private well water annually (to include nitrates, coliform bacteria, total dissolved solids and pH). Resources and information regarding private well testing including any additional testing applicable to local/area conditions are typically available from local and state health departments. If contaminated well water is suspected, an alternate water source should be used until testing results are available.

If the cyanosis is due to a congenital methemoglobinemia, it could be from inheritance of HbM (can be detected by hemoglobin electrophoresis) or from inherited methemoglobin reduction system defects (such as NADH-dependent methemoglobin reductase deficiency which can be detected by enzyme analysis).

More detailed information regarding this answer can be found in the "Who Is at Risk of Adverse Health Effects from Overexposure to Nitrates and Nitrites?", "What Are the Health Effects from Exposure to Nitrates and Nitrites" and "How Should Patients Potentially Overexposed to Nitrates and Nitrites Be Evaluated (Clinical Assessment)?" sections.

4. Questions that may help determine the cause of the infant's cyanosis include

   - Where is the home located?
   - What activities have been occurring around the home?
   - What type of sewer system connects to the home?
   - What are family members' occupations, avocations, and hobbies?
   - What is the source of the family's drinking water and how is it supplied?
   - What is used for home heating and cooking? (i.e., central heating [gas/electric], fireplace [wood burning/gas], portable heater [gas/

electric], stove [wood burning/gas/electric and if properly vented], use of emergency generators).

Information to gather from families with infants includes

- The type of formula, feeding regimen, and source of dilution water;
- The infant's history of recent gastroenteritis; and
- Family history, including recent use of all medications by both infant and mother.

For information on taking a complete exposure history including questions to ask adults and children, see

ATSDR Case Studies in Environmental Medicine: Taking an Exposure History
https://www.atsdr.cdc.gov/csem/csem.asp?csem=33&po=0

and

ATSDR Case Studies in Environmental Medicine: Taking a Pediatric Exposure History
http://www.atsdr.cdc.gov/csem/csem.asp?csem=26&po=0

More detailed information regarding this answer can be found in the "How Should Patients Potentially Overexposed to Nitrates or Nitrites Be Evaluated (Clinical Assessment)?" section.

5. Causes of high nitrate concentrations in well water include runoff from the use of nitrogen-containing agricultural fertilizers (including anhydrous ammonia) and seepage of organic nitrogen-containing material from animal wastes or septic sewer systems.

More detailed information regarding this answer can be found in the "Where Are Nitrates and Nitrites Found?" section.

6. The well water should be tested for nitrate concentration, the presence of coliform bacteria, total dissolved solids and pH (this is what EPA recommends for annual private well water testing). The family can contact the local or state health department to perform or suggest contractors that can run these and any other tests applicable to local/area conditions. It is most important to identify the source of the methemoglobin-inducing agent and to preclude any further exposure. If nitrate-contaminated well water is the source, you should recommend using an alternative water source to dilute infant formula.

   More detailed information regarding this answer and private well water testing/maintenance can be found in the "Who Is at Risk of Adverse Health Effects from Overexposure to Nitrates and Nitrites?" and "What Instructions Should Be Given to Patients to Prevent Overexposure to Nitrates and Nitrites?" sections.

7. Infants younger than 4 months of age are more susceptible to developing methemoglobinemia for a number of reasons including:

   - HbF
     - A large proportion of hemoglobin in young infants is in the form of fetal hemoglobin. Fetal hemoglobin (HbF) is more readily oxidized to MetHb by nitrites than is adult hemoglobin.
   - Impaired reduction of MetHb:
     - At birth, NADH-dependent methemoglobin reductase (also called cytochrome-b5 reductase), the enzyme responsible for reduction of induced methemoglobin back to normal hemoglobin, has only about half the activity it has in adults.
   - Infant gut pH

- Infant gut pH is normally higher than in older children and adults. The higher gastric pH enhances bacterial growth in the infant intestinal tract involved in conversion of ingested nitrate to the more potent nitrite (which acts as a potent oxidizing agent).

- Other factors

  - Gastroenteritis can increase in vivo transformation of nitrate to nitrite and systemic absorption of nitrite from the large intestine.
  - Young infants can develop methemoglobinemia with systemic metabolic acidosis. The systemic metabolic acidosis is often caused by dehydration associated with diarrhea or sepsis, but it can occur with renal disorders as well.

More detailed information regarding this answer can be found in the "Who Is at Risk of Adverse Side Effects from Overexposure to Nitrates and Nitrites?", "What Is the Biologic Fate of Nitrates and Nitrites in the Body?" and "What Are the Health Effects from Exposure to Nitrates and Nitrites?" sections.

8. The most common cause of a poor response to methylene blue treatment is unrecognized G6PD deficiency.

   More detailed information regarding this answer can be found in the "How Should Patients Potentially Overexposed to Nitrates and Nitrites Be Treated and Managed?" section.

9. Treatment options for patients with G6PD deficiency might include exchange transfusion and/or hyperbaric oxygen therapy.

   More detailed information regarding this answer can be found in the "How Should Patients Potentially Overexposed to Nitrates and Nitrites Be Treated and Managed?" section.

What Are Nitrates and Nitrites?

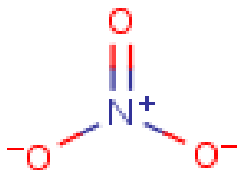
Learning Objective	Upon completion of this section, you will be able to • Describe what nitrates and nitrites are.
Introduction	Nitrates and nitrites can be categorized into inorganic and organic forms based on their chemical structure. There are similarities and differences between these two chemical forms that affect their pharmacokinetic and pharmacodynamic properties and their subsequent biologic effects in humans. This course will focus on inorganic nitrates.
Inorganic Nitrates and Nitrites	Inorganic nitrate (NO_3^-) and nitrite (NO_2^-) are water soluble (as a result of their interaction with the positively charged portions of polar water molecules) (Figure 1) and commonly exist as salts of nitric acid and nitrous acid, respectively. They are often bound to a metal cation such as Na+ or K+ and occur naturally through the fixation of atmospheric nitrogen and oxygen as part of the environmental nitrogen cycle (the cyclic movement of nitrogen in different chemical forms from the environment, to organisms, and then back to the environment as illustrated in Figure 2). Inorganic nitrites are also produced endogenously through oxidation of nitrous oxide (NO) formed from the enzymatic degradation of L-arginine and through the reduction of nitrate with xanthine oxidoreductase [Omar et al. 2012; Jansson et al. 2008; Rhodes et al. 1995; Leaf et al. 1989; Green et al. 1981].

Organic Nitrates and Nitrites

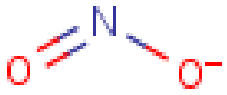
The organic forms of nitrates and nitrites are more complex and most are synthesized medicinal products (except ethyl nitrite) [Omar et al. 2012]. See Table 2. Organic nitrates are small non-polar hydrocarbon chains attached to a nitrooxy-radical (-ONO_2; -ONO for amyl and ethyl nitrite). The addition of aliphatic or aromatic groups of variable length and volume affect the lipophilic properties of these molecules [Thatcher et al. 2004]. It has been suggested that for some molecules, the greater the number of –ONO_2 groups, the greater its potency [Wenzel et al 2007]; (the potency being dependent on the molecule's lipophilicity) [Schuhmacher et al. 2009; Koenig et al. 2007].

Structures of Nitrate and Nitrite Ions

Figure 1. Structures of Nitrate and Nitrite Ions



Nitrate (CAS: registry number: 14797-55-8)



Nitrite (CAS Registry Number: 14797-65-0)

Figure 1. Adapted from [ATSDR 2006 Appendix E].

Key Points	• Nitrates and Nitrites exist in organic and inorganic forms. • The chemical form affects the pharmacokinetic and pharmacodynamic properties of nitrates and nitrates. • Inorganic nitrates and nitrites are generally more water soluble than organic nitrates and nitrates. • Inorganic nitrates and nitrites are produced endogenously and exogenously. • Organic nitrates and nitrites are mostly synthesized medicinal products. • Organic nitrates and nitrites are generally more complex and lipophilic than inorganic nitrates and nitrites.

Progress Check

1. Which of the following is false regarding inorganic nitrites and nitrates?

   A. Are naturally occurring inorganic ions.
   B. Are relatively insoluble in water.
   C. Are produced exogenously and endogenously.
   D. Generally have different pharmacokinetic properties than organic forms.

   To review relevant content, see "Inorganic Nitrates and Nitrites" in this section.

2. Which of the following is true regarding nitrates and nitrites?

   A. Both forms have the same pharmacodynamic properties.
   B. Organic forms are naturally occurring from the fixation of nitrogen in the environment.
   C. Inorganic forms are mostly synthesized medicinal products.
   D. Inorganic forms are mostly water soluble.

   To review relevant content, see "Inorganic Nitrates and Nitrites" and "Organic Nitrates and Nitrites" in this section.

Where Are Nitrates and Nitrites Found?

Learning Objective

Upon completion of this section, you will be able to

- Identify sources of nitrates and nitrites.

Introduction	Understanding the environmental fate of nitrates and nitrites may help pinpoint potential sources of exposure. This would be important in assessment of patient exposure risk, prevention and mitigation of nitrate/nitrite overexposure and in the prevention of adverse health effects from exposure.
Environmental Nitrogen Cycle	In general, the following describes the activity of nitrates and nitrites in the environment (as illustrated in Figure 2). Microbial action in soil or water decomposes wastes containing organic nitrogen into ammonia, which is then oxidized to nitrite and nitrate. • Because nitrite is easily oxidized to nitrate, nitrate is the compound predominantly found in groundwater and surface waters. • Contamination with nitrogen-containing fertilizers (e.g. potassium nitrate and ammonium nitrate), or animal or human organic wastes, can raise the concentration of nitrate in water. • Nitrate-containing compounds in the soil are generally water soluble and readily migrate with groundwater [ATSDR 2006; EPA 2004; Mackerness and Keevil 1991; Shuval and Gruener 1992].

The Nitrogen Cycle

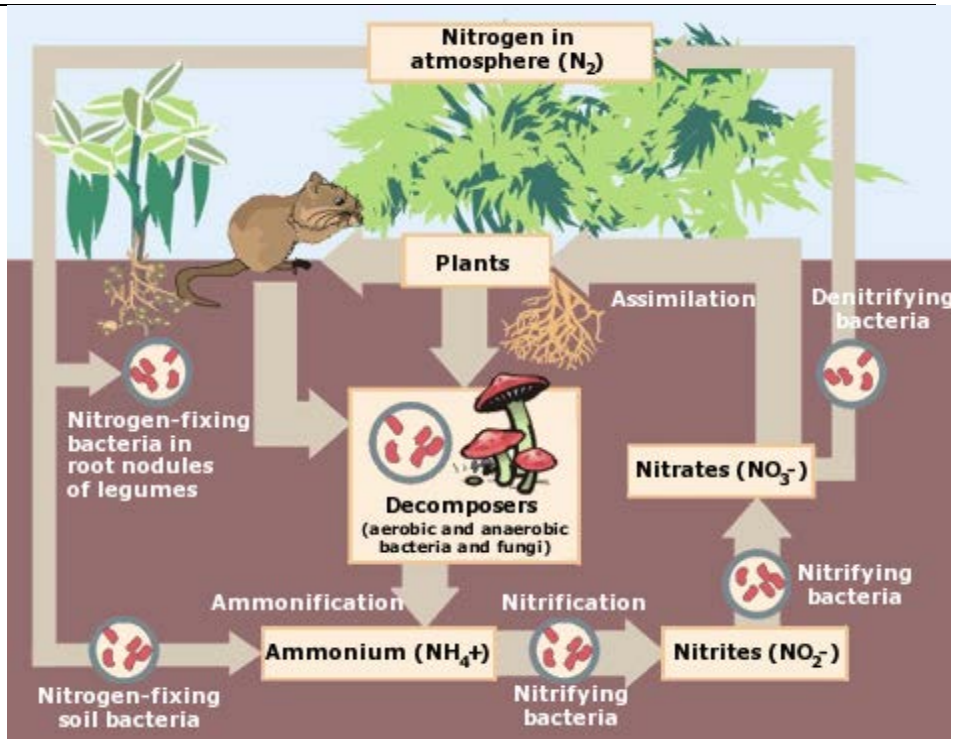


Figure 2. The Nitrogen Cycle

From US EPA
http://www.epa.gov/caddis/ssr_amm_nitrogen_cycle_popup.html

Water Contamination	Shallow, rural domestic wells are those most likely to be contaminated with nitrates, especially in areas where nitrogen-based fertilizers are widely used [Dubrovsky and Hamilton 2010; NRC 1995]. • Approximately 15 percent of Americans rely on their own private drinking water supplies which are not subject to U.S. Environmental Protection Agency (EPA) standards, although some state and local governments do set guidelines to protect users of these wells [Census Bureau 2011 and 2012]. • In agricultural areas, nitrogen-based fertilizers are a major source of contamination for shallow groundwater aquifers that provide drinking water [Dubrovsky and Hamilton 2010; CDC 1995]. • A recent United States Geological Survey study showed that 7 percent of 2,388 domestic wells and about 3 percent of 384 public-supply wells nationwide were contaminated with nitrate levels above the EPA drinking water standard of 10 parts per million (ppm) or 10 mg/L [Dubrovsky and Hamilton 2010]. • Elevated concentrations were most common in domestic wells that were shallow (less than 100 feet deep) and located in agricultural areas because of relatively large nitrogen sources, including septic systems, fertilizer use, and livestock [Dubrovsky and Hamilton 2010]. • Although suppliers of public water sources are required to monitor nitrate concentrations regularly, few private rural wells are routinely tested for nitrates [EPA 1990a; EPA 2007; CDC 2009]. • During spring melt or drought conditions, both domestic wells and public water systems using surface water can show increased nitrate levels [Nolan et al. 2002; Dubrovsky and Hamilton 2010]. • Drinking water contaminated by boiler fluid additives may also contain increased levels of nitrites [CDC 1997]. • Mixtures of nitrates/nitrites with other well contaminants such as pesticides and VOCs have been reported [Squillace et al. 2002].

Food Contamination	Nitrate and nitrite overexposure has been reported via ingestion of foods containing high levels of nitrates and nitrites. Inorganic nitrates and nitrites present in contaminated soil and water can be taken up by plants, especially green leafy vegetables and beet root [Butler and Feelisch 2008]. • Contaminated foodstuffs, prepared baby foods, and sausage/meats preserved with nitrates and nitrites have caused overexposure in children [Savino et al. 2006; Greer and Shannon 2005; Sanchez-Echaniz et al. 2001; Dusdieker et al. 1994; Rowley 1973]. • Although vegetables are seldom a source of acute toxicity in adults, they account for about 80% of the nitrates in a typical human diet [Hord 2011; Pennington 1998]. • Celery, spinach lettuce, red beetroot and other vegetables (See Table 1) have naturally greater nitrate content than other plant foods do [Hord 2011; EFSA 2008; Keating et al. 1973; Vittozzi 1992]. • The remainder of the nitrate in a typical diet comes from drinking water (about 21%) and from meat and meat products (about 6%) in which sodium nitrate is used as a preservative and color-enhancing agent [Alexander et al. 2010; Gilchrist et al. 2010; Lundberg et al. 2009; Lundberg et al. 2008; Norat et al. 2005; Chan 1996; Saito et al. 2000]. • For infants who are bottle-fed, however, the major source of nitrate exposure is from contaminated drinking water used to dilute formula [Hord et al. 2010; EPA 2007]. • Bottled water is regulated by the U.S. Food and Drug Administration (FDA) as a food. It is monitored for nitrates, nitrites and total nitrates/nitrites.

Nitrate Content of Selected Vegetables

Table 1. Nitrate Content of Selected Vegetables
[Adapted from Hord et al. 2011; Santamaria 2006]

Vegetable	**Nitrate content, mg/100g fresh weight**
Celery, lettuce, red beetroot, spinach	Very High (> 2500)
Parsley, leek, endive, Chinese cabbage, fennel	High (100-250)
Cabbage, dill, turnip	Medium (50-100)
Broccoli, carrot, cauliflower, cucumber, pumpkin	Low (20-50)
Artichoke, asparagus, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon	Very Low (<20)

Other Sources of Exposure	Nitrate or nitrite exposure can occur from certain medications and volatile nitrite inhalants. Accidental and inadvertent exposures to nitrites as well as ingestion in suicide attempts have been reported [Aquanno et al. 1981; Gowans 1990; Ellis et al. 1992; Bradberry et al. 1994; Saito et al. 1996 and 2000; EPA 2007; Harvey et al. 2010]. Deliberate abuse of volatile nitrites (amyl, butyl, and isobutyl nitrites) frequently occurs [Wu et al. 2005; Lacy and Ditzler 2007]. Amyl nitrite (nicknamed by some as “poppers”) is used commercially as a vasodilator and butyl/isobutyl nitrites can be found in products such as room air fresheners [Kurtzman et al. 2001; Hunter et al. 2011]. Fatalities have been reported in adults exposed to nitrates in burn therapy [Kath et al 2011]; however infants and children are especially susceptible to adverse health effects from exposure to topical silver nitrate used in burn therapy [Cushing et al. 1969; Chou et al. 1999; Nelson and Hostetler 2003]. Other medications implicated in methemoglobinemia include • Quinone derivatives (antimalarials), • Nitroglycerine, • Bismuth subnitrite (antidiarrheal), • Ammonium nitrate (diuretic), • Amyl and sodium nitrites (antidotes for cyanide and hydrogen sulfide poisoning), • Isosorbide dinitrate/tetranitrates (vasodilators used in coronary artery disease therapy), • Benzocaine (local anesthetic), and • Dapsone (antibiotic). Other possible sources of exposure include ammonium nitrate found in cold packs and nitrous gases used in arc welding. An ethyl nitrite folk remedy called “sweet spirits of nitre” has caused fatalities [Coleman and Coleman 1996; Dusdieker and Dungy 1996].

Key Points

- Shallow, rural domestic wells are those most likely to be contaminated with nitrates, especially in areas where nitrogen based fertilizers are in widespread use.
- Other nitrate sources in well water include seepage from septic sewer systems and animal wastes.
- Foodstuffs high in nitrates, home prepared baby foods, and sausage/meats preserved with nitrates and nitrites have caused overexposure in children.
- Nitrate or nitrite exposure can occur from certain medications and volatile nitrite inhalants.

Progress Check

3. Which of the following is/are true regarding nitrites and nitrates in the environment?

   A. Nitrate is the form predominately found in groundwater and surface waters.
   B. Nitrate containing compounds in the soil are generally water soluble.
   C. Nitrates readily migrate with ground water.
   D. All of the above.

   To review relevant content, see "Environmental Nitrogen Cycle" in this section.

4. Which of the following water sources is generally most likely to contain high levels of nitrates or nitrites?

   A. Bottled water.
   B. Large municipal water supplies.
   C. Shallow, rural domestic wells.
   D. Water from deep wells.

   To review relevant content, see "Water Contamination" in this section.

5. Medications which have been implicated in nitrate/nitrite toxicity include

   A. Nitroglycerin.
   B. Bismuth subnitrite (antidiarrheal).
   C. Silver nitrate burn cream.

D. All of the above.

To review relevant content, see "Other Sources of Exposure" in this section.

What Are Routes of Exposure to Nitrates and Nitrites?

Learning Objective	Upon completion of this section, you will be able to • Describe primary routes of exposure to nitrates and nitrites.
Introduction	The primary routes of exposure to nitrates and nitrites may differ depending on occupational and non-occupational factors. Non-occupational factors may include • Age, • Diet, • Medications, • Hobbies (such as gardening, arc welding, etc.), • History of inhalational drug use, • Source of drinking/cooking water and how it is supplied, • Outdoor activities, as well as • The chemical form of the nitrates and nitrites.
Occupational and Paraoccupational Exposures	Occupational exposure occurs primarily through the inhalation and dermal routes. Explosive and fertilizer industry workers may be exposed to nitrate through inhalation of dusts containing nitrate salts. Dusts can also dissolve in sweat exposing skin to concentrated solutions of the salts. Farmers may experience periodic exposures depending on their activities, especially with regard to the handling of fertilizers. Exposure of family members to nitrates from dusts brought home on work clothes has been reported [Rosenman 2007].

Non-occupational Exposures	The primary route of non-occupational exposure is ingestion of water or foodstuffs that contain high levels of nitrates or nitrites. Inhalation exposures may occur from inhalant drug use and dermal exposures may occur from some topical medications. These would be special instances and not the primary routes of exposure for the general population.
Key Points	• Primary occupational routes of exposure to nitrates and nitrites include inhalation and dermal routes. • The primary route of exposure to nitrates and nitrites for the general population is ingestion. • Inhalation and dermal exposures have been reported in non-occupational settings under certain circumstances, but are not the primary routes of exposure for the general population.
Progress Check Questions	6. Which of the following is true regarding route(s) of exposure to nitrates and nitrites in humans? A. The primary route of occupational exposure is ingestion. B. The primary route of exposure for the general population is dermal. C. Primary routes of exposure are the same for occupational and non-occupational populations. D. None of the above. *To review relevant content, see "Occupational and Paraoccupational Exposures" and "Non-occupational Exposures" in this section.*

Who Is at Most Risk of Adverse Health Effects from Overexposure to Nitrates and Nitrites?

Learning Objective	Upon completion of this section, you will be able to • Identify the population most susceptible to the adverse health effects from overexposure to nitrates and nitrites.

Introduction	Infants less than 4 months of age are most at risk of adverse health effects from over exposure to nitrates and nitrites through ingestion of formula diluted with nitrate contaminated water [EPA 2007; WHO 2011a; WHO 2011b]. Although there is no nutritional indication to add complementary foods to the diet of a healthy term infant before 4 to 6 months of age, the American Academy of Pediatrics suggests that home-prepared infant foods from vegetables (i.e. spinach, beets, green beans, squash, carrots) should be avoided until infants are 3 months or older [Greer and Shannon 2005]. Gastroenteritis with vomiting and diarrhea can exacerbate nitrite formation in infants and has been reported to be a major contributor to methemoglobinemia risk in infants independent of nitrate/nitrite ingestion [Lebby et al. 1993; Gebara and Goetting 1994; Avery 1999; Nelson and Hostetler 2003; DeBaun et al. 2011]. In addition, the pregnant woman and her fetus might be more sensitive to toxicity from nitrites or nitrates at or near the 30th week of pregnancy [Gitto et al. 2002; Gordon 2012]. Individuals with glucose-6-phsphate dehydrogenase (G6PD) deficiency may have greater susceptibility to the oxidizing effects of methemoglobinemia inducers.

Infants Are at Highest Risk

Infants younger than 4 months of age who are fed formula diluted with water from untested rural domestic wells are especially prone to developing health effects from nitrate exposure [EPA 2007; WHO 2011a; WHO 2011b; Dusdieker and Dungy 1996]. They are more susceptible to developing methemoglobinemia for a number of reasons including:

Infant gut pH

- The high pH of the infant gastrointestinal system favors the growth of nitrate-reducing bacteria [Kross et al. 1992; Nelson and Hostetler 2003], particularly in the stomach and especially after ingestion of contaminated waters. The stomach of adults is typically too acidic to allow for significant bacterial growth and the resulting conversion of nitrate to nitrite.

HbF

- A large proportion of hemoglobin in young infants is in the form of fetal hemoglobin. Fetal hemoglobin (HbF) is more readily oxidized to MetHb by nitrites than is adult hemoglobin [Rehman 2001; Nelson and Hostetler 2003]. Over time, adult forms of hemoglobin gradually increase and HbF decreases [McKenzie 2010]. Infants with a higher proportion of fetal hemoglobin may have severely reduced oxygenation before cyanosis appears clinically [Steinhorn 2008]. Therefore, infants, especially premature ones, are particularly susceptible.

Impaired reduction of MetHb

- At birth, NADH-dependent methemoglobin reductase (also called cytochrome-b5 reductase), the major enzyme responsible for reduction of induced methemoglobin back to normal hemoglobin, has only about half the activity it has in adults [Hjelt et al. 1995; ATSDR 2004; Smith 1991; Nelson and Hostetler 2003; McKenzie 2010]. The level of cytochrome-b5 reductase does not reach adult levels

until at least 4 months of age [Lebby et al. 1993; Nelson and Hostetler 2003].

Other factors

Infection and inflammatory reactions can increase endogenous synthesis of nitrate in both infants and adults [NRC 1995; Nelson and Hostetler 2003].

- Gastroenteritis with vomiting and diarrhea can exacerbate nitrite formation in infants. This has been reported as a major contributor to MetHb risk in infants independent of nitrate/nitrite ingestion [Lebby et al. 1993; Gebara and Goetting 1994; Avery 1999; Nelson and Hostetler 2003].
- Gastroenteritis can increase the in vivo transformation of nitrate to nitrite and systemic absorption of nitrite from the large intestine.
- Young infants can develop methemoglobinemia with systemic metabolic acidosis. The systemic metabolic acidosis is often caused by dehydration associated with diarrhea or sepsis, but it can occur with renal disorders as well [Nelson and Hostetler 2003; Hanukoglue and Danon 1996; Sager et al. 1995]. With sepsis, it is thought that nitric oxide is released and oxidizes hemoglobin as it is reduced to nitrate [Nelson and Hostetler 2003; Ohashi et al. 1998]. With acidosis, the NADH methemoglobin reductase system is affected leading to as much as 50% decrease in methemoglobin reduction [Nelson and Hostetler 2003].

These factors combine to place young infants with diarrhea, who are fed formula diluted with nitrate-contaminated well water, at the greatest risk for toxicity [Johnson and Kross 1990; Zeman et al. 2002; EPA 2007; WHO 2011a, 2011b].

Pregnancy	The pregnant woman and her fetus represent another high-risk group. Pregnancy is a high oxygen demand physiologic state. Due to the increased intake and utilization of oxygen, increased levels of oxidative stress are reasonably expected. The hematologic changes of pregnancy include a 40-50% increasing blood volume (plasma greater than RBC mass) expansion peaking at around 30 weeks [Gordon 2012]. With plasma volume increasing more than the RBC mass, the maternal hematocrit falls resulting in a "physiologic anemia of pregnancy" reaching a peak at 30 to 34 weeks [Gordon 2012]. Due to oxidative stress, methemoglobin is continually produced within red blood cells, but its levels are kept low (0.5% to 2.5% of total hemoglobin) by enzymatic pathways that work to reduce methemoglobin. Conditions such as pregnancy with its high oxygen demand and increased levels of oxidative stress may overwhelm the body's ability to reconvert methemoglobin back to hemoglobin, resulting in increased methemoglobin levels [Gitto et al. 2002]. Exposure to nitrates also increases oxidative stress and depletes antioxidant reserves. Thus, pregnant women may be more sensitive to the induction of clinical methemoglobinemia by nitrites or nitrates at or near the 30th week of pregnancy when oxidative stress peaks. Reproductive outcome studies performed at sites with high nitrate levels in the water supply provide some evidence of maternal transfer of nitrate and nitrite [Manassaram et al. 2006; Tabacova et al. 1997 and 1998; Croen et al. 2001].

Others with Increased Risk	An increased risk of developing methemoglobinemia from exposure to oxidizing agents has been reported in individuals with coexisting • Anemia, cardiovascular disease, lung disease, sepsis • Glucose-6-phosphate deficiency (more common in individuals of African, Asian or Mediterranean descent) • Metabolic problems with pyruvate kinase and RBC methemoglobin reductase • Presence of other abnormal hemoglobin species (structural abnormalities of the hemoglobin molecule itself) including carboxyhemoglobin, sulfhemoglobin and sickle hemoglobin (HbS) [Ash-Bernal et al. 2004; Skold et al. 2011] Genetic factors may increase the risk of drug induced methemoglobinemia and hemolytic anemia [McDonagh et al. 2013]. Recreational drug users are at increased exposure risk, especially users of volatile nitrite inhalers and drugs like cocaine. Cocaine can be adulterated with a variety of substances including phenacetin and local anesthetics like benzocaine [Hunter et al 2011; Flomenbaum et al. 2006] (see Table 2).
Key Points	• Infants younger than 4 months of age are most at risk of developing adverse health effects from overexposure to nitrates and nitrites through ingestion of formula diluted with nitrate contaminated water. • Gastroenteritis with vomiting and diarrhea can exacerbate nitrite formation in infants and has been reported to be a major contributor to MetHb risk in infants independent of nitrate/nitrite ingestion. • The pregnant woman and her fetus might be more sensitive to toxicity from nitrites or nitrates at or near the 30th week of pregnancy when oxidative stress peaks. • Populations that may become symptomatic at lower levels of MetHb than predicted include patients with

	oxygen transport or delivery conditions like anemia, cardiovascular disease, lung disease, sepsis and presence of other structural hemoglobin variants. • Other conditions that increase the risk of developing methemoglobinemia include enzyme deficiencies such as G6PD deficiency and RBC methemoglobin reductase deficiency/impairment as well as other genetic factors.
Progress Check	7. High risk populations for nitrate/nitrite toxicity include A. Infants younger than 4 months old. B. Infants with diarrhea or vomiting. C. Infants fed formula diluted with untested private well water. D. All of the above. *To review relevant content, see "Infants Are at Highest Risk" in this section.*

What Are U.S. Standards and Regulations for Nitrates and Nitrites Exposure?

Learning Objectives	After completing this section, you will be able to • Describe the U.S. Environmental Protection Agency's (EPA's) recommended limit for nitrates and nitrites in drinking water. • Describe the U.S. Food and Drug Administration's (FDA's) recommended limit for nitrates and nitrites in bottled water and foodstuffs.
Introduction	EPA has set an enforceable standard called a maximum contaminant level (MCL) in water for nitrates at 10 parts per million (ppm) (10 mg/L) and for nitrites at 1 ppm (1 mg/L) [EPA 2002; EPA 2012]. • EPA believes that exposure below this level is not expected to cause significant health problems. • All public water supplies must abide by these regulations.

	• Given present technology and resources, this MCL is also a level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water. Once a water source is contaminated, the costs of protecting consumers from nitrate exposure can be significant. This is because: • Nitrate is not removed by conventional drinking water treatment processes, and • Its removal requires additional, relatively expensive treatment units [EPA 2004].
Intake Limits	The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the United Nations/World Health Organization and the European Commission's Scientific Committee on Food have set an acceptable daily intake (ADI) for nitrate of 0–3.7 milligrams (mg) nitrate ion/kilogram (kg) body weight. This intake appears to be safe for healthy neonates, children, and adults. The same is also true of the EPA reference dose (RfD) for nitrate of 1.6 mg nitrate nitrogen/kg body weight per day (equivalent to about 7.0 mg nitrate ion/kg body weight per day) [EPA 2002; EPA 2012]. JECFA has proposed an ADI for nitrite of 0–0.07 mg nitrite ion/kg body weight. EPA has set an RfD of 0.l mg nitrite nitrogen/kg body weight per day (equivalent to 0.33 mg nitrite ion/kg body weight per day) [Mensinga et al. 2003; Abadin et al. 1998; EPA 2002; EPA 2012].

Bottled Water and Food Additives Limits

The FDA regulates allowable levels of inorganic nitrate and nitrite in bottled water [FDA 2005] as well as levels allowable in foodstuffs [FDA 2003].

The FDA's bottled water standard is based on the EPA standards for tap water. The bottled water industry must also follow FDA's Current Good Manufacturing Practices (CGMPs) for processing and bottling drinking water. If these standards are met, water is considered safe for most healthy individuals. However, although not often reported, bottled water outbreaks do occur. More information on bottled water can be found at http://www.cdc.gov/healthywater/drinking/bottled/index.html

Bottled water

Allowable levels in bottled water:

- Nitrate 10 mg/L (as nitrogen)
- Nitrite 1 mg/L (as nitrogen)
- Total nitrates, nitrites 10 mg/L (as nitrogen)

Allowable levels as an additive to foods:

- As a preservative and color fixative, with or without sodium nitrite, in

  - Smoked, cured sablefish
  - Smoked, cured salmon
  - Smoked, cured shad

  so that the level of sodium nitrate does not exceed 500 parts per million (ppm) and the level of sodium nitrite does not exceed 200 ppm in the finished product.

- As a preservative and color fixative, with or without sodium nitrite, in meat-curing preparations for the home curing of meat and meat products (including poultry and wild game), with directions for use which limit the amount of sodium nitrate to not more than 500 ppm in the finished meat product

	and the amount of sodium nitrite to not more than 200 ppm in the finished meat product. • The food additive potassium nitrate may be safely used as a curing agent in the processing of cod roe, in an amount not to exceed 200 ppm of the finished roe. The U.S. Department of Agriculture's (USDA's) Food Safety and Inspection Service (FSIS) regulates food ingredients approved for use in the production of meat and poultry products. This includes inspection for required labeling of meat products when substances such as sodium nitrate are used in meat packaging [USDA 2012].
Environmental Standards	The current water standard for nitrate is based on levels considered low enough to protect infants from methemoglobinemia. • Some published results suggest a possible association between nitrate exposure during pregnancy and human malformations [Croen et al. 2001; Brender et al. 2004; Brender et al. 2011]. • However, a review of the toxicology in relation to possible adverse effects on reproduction and development offers no evidence for teratogenic effects attributable to nitrate or nitrite ingestion [Manassaram et al. 2006; Huber et al. 2013]. • The present maximum contaminant level appears to adequately protect even sensitive populations from nitrate-induced toxicity [Fan and Steinberg 1996; EPA 2006]. • EPA concludes that the evidence in the literature showing an association between exposures to nitrate or nitrites and cancer in adults and children is conflicting [EPA 1991, 2002, 2006].
Key Points	• The current water standard for nitrate is based on protection of infants from methemoglobinemia. • In vivo conversion of nitrates to nitrites significantly enhances nitrates' toxic potency.

Progress Check

8. EPA's MCL for nitrates in drinking water is based on which of the following?

   A. Protection of general public from reproductive and developmental health effects.
   B. Protection of infants from methemoglobinemia.
   C. Protection of the general population from cancer endpoints seen in exposed workers.
   D. Protection of the general public from cardiovascular health effects.

   To review relevant content, see "Environmental Standards" in this section.

9. Which of the following is true regarding U.S. standards and regulations of nitrate and nitrite levels?

   A. FDA regulates levels of nitrates and nitrites as additives to food.
   B. FDA regulates levels of nitrates and nitrites in fresh fruits and vegetables.
   C. EPA regulates levels of nitrates and nitrites in bottled water.
   D. EPA regulates the level of nitrates and nitrites in private wells.
   E. All of the above
   F. None of the above.

   To review relevant content, see "Environmental Standards" in this section.

What Is the Biologic Fate of Nitrates and Nitrites in the Body?

Learning Objectives

Upon completion of this section, you will be able to

- Describe what happens to nitrates and nitrites once they enter the body.

Introduction	Exposure to nitrates and nitrites may come from both internal nitrate production and external sources. Intake of some amount of nitrates is a normal part of the nitrogen cycle in humans. The mean intake of nitrate per person in the United States is about 40–100 milligrams per day (mg/day) (in Europe it is about 50–140 mg/day). Nitrate can be synthesized endogenously from nitric oxide (especially in the case of inflammation), which reacts to form nitrite [Hord 2011; ATSDR 2004; Mensinga et al. 2003]. Figure 3 shows ways that nitrate, nitrite and nitric oxide can be produced and utilized from exogenous and endogenous sources [Hord et al 2009; Hord 2011].
Absorption	In the proximal small intestine, nitrate is rapidly and almost completely absorbed (bioavailability at least 92%) [Mensinga et al. 2003; Carlsson et al. 2001]. • Inorganic nitrate/nitrite can be absorbed via inhalation [Holmes et al 2005; Gladwin et al. 2004]. • Inorganic nitrate/nitrite does not undergo first pass metabolism [Pannala et al. 2003; Omar et al. 2012].
Distribution	Inorganic nitrates/nitrites are distributed widely through the circulation with approximately 25% of absorbed nitrate concentrating in the salivary glands [Carlsson et al. 2001]. Salivary, plasma, and urinary levels of nitrate and then nitrite rise abruptly after ingestion [Doel et al. 2005; Duncan et al. 1995; Walker 1996]. An increase in inorganic nitrite levels peaks around 3 hours post ingestion and can be detected about an hour after ingestion [Hunault et al. 2009; Gago et al. 2008].

Metabolism of Inorganic Nitrates and Nitrites	The two main metabolic pathways for inorganic nitrates/nitrites are 1. The nitrate-nitrite-NO pathway (Figure 3) and 2. Enterosalivary circulation pathway (nitrate reductase activity of bacteria on the tongue generates nitrite and nitrite which is metabolized to NO in the stomach and circulation) [Hord 2011]. Approximately 5%–10% of the total nitrate intake is converted to nitrite by bacteria in the saliva, stomach, and small intestine [Hord 2011]. • In vivo conversion of nitrates to nitrites significantly enhances nitrates' toxic potency. • This reaction is pH dependent, with no nitrate reduction occurring below pH 4 or above pH 9. • The high pH of the infant gastrointestinal system makes them more susceptible to nitrite toxicity from elevated nitrate/nitrite ingestion. The metabolic pathway of plasma and tissue nitrates depends on local conditions such as tissue oxygenation, and inflammatory state. In the skin, local conditions also include ultraviolet light exposure [Mowbray et al. 2009; Oplander et al. 2009]. Nitrate can be reduced to nitrite and nitric oxide when needed physiologically or as part of pathological processes (see Figure 3 [Hord 2011; van Fassen et al. 2009; Weitzberg et al. 2010; Kapil et al. 2010; Panesar 2008; Rocha et al. 2011; Webb et al. 2008; Jiang et al. 2008]. Mammalian metalloproteins and enzymes that have nitrate reductase activity include aldehyde oxidase, heme proteins, mitochondria and xanthine reductase [Hord 2011; Larsen et al. 2011; Jansson et al. 2008]. The reaction of nitrite with endogenous molecules to form N-nitroso compounds may have toxic or carcinogenic effects [ATSDR 2004; Powlson et al. 2008; Rao et al. 1982].

Excretion	• Approximately 60% to 70% of an ingested nitrate dose is excreted in urine within the first 24 hours [Carlsson et al. 2001]. • About 25% is excreted in saliva through an active blood nitrate transport system and potentially is reabsorbed [Doel et al. 2005; Hord 2011]. • Half-lives of parent nitrate compounds are usually less than 1 hour; half-lives of metabolites range from 1 hour to 8 hours [Walker 1996; EPA 1990b]. • In the Fourth National Report on Human Exposure to Environmental Chemicals, urinary levels of nitrate were measured in a subsample of the National Health and Nutrition Examination Survey (NHANES) consisting of participants aged 6 years and older during 2007-2008. The geometric mean for urinary nitrate (in mg/g of creatinine) for the US population aged 6 years and older during 2007-2008 was 47.7, with a 95% confidence interval of 45.9-49.7 [CDC 2013]. Note that these measurements are used in population based public health research and not intended for clinical decision making on individual patients.

Figure 3

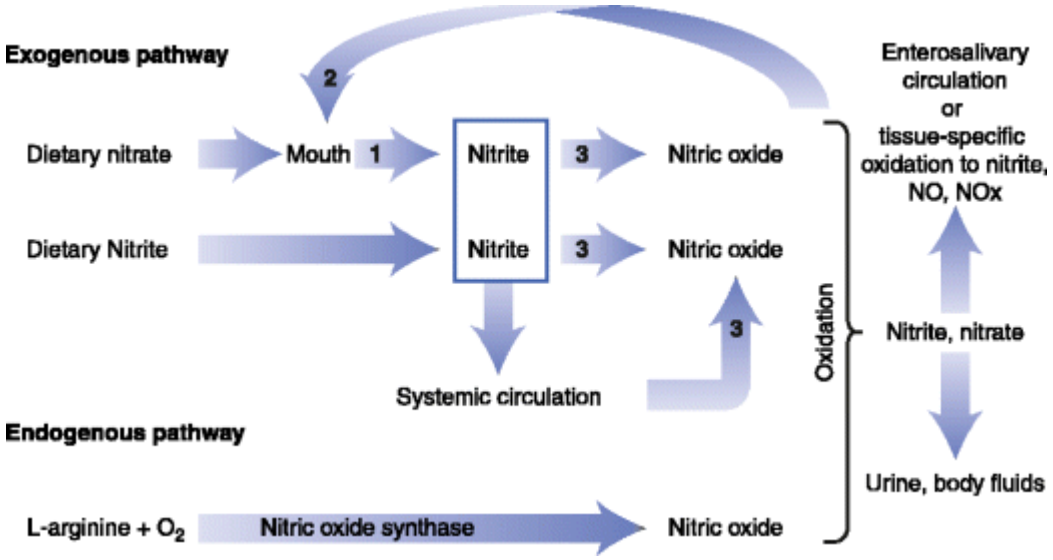


Fig. 3 A schematic diagram of the physiologic disposition of nitrate, nitrite, and nitric oxide (NO) from exogenous (dietary) and endogenous sources. The action of bacterial nitrate reductases on the tongue and mammalian enzymes that have nitrate reductase activity in tissues are noted by the number 1. Bacterial nitrate reductases are noted by the number 2. Mammalian enzymes with nitrite reductase activity are noted by the number 3 [Adapted from Hord et al. 2009].

Key Points

- Exposure to nitrate and nitrites may come from both internal nitrate production and external sources.
- Intake of some amount of nitrates is a normal part of the nitrogen cycle in humans.
- Nitrate can be reduced to nitrite and nitric oxide when needed physiologically or as part of pathological processes depending on local conditions such as inflammation and tissue oxygenation.
- In vivo conversion of nitrates to nitrites significantly enhances nitrates' toxic potency.
- Approximately 5%–10% of the total nitrate intake is converted to nitrite by bacteria in the saliva, stomach, and small intestine.
- 60-70% of an ingested nitrate dose is excreted in urine within 24 hours.

Progress Checks

10. The toxicity of nitrates is enhanced by in vivo conversion to

    A. Urea.
    B. CO_2.
    C. Protein.
    D. Nitrites.

    To review relevant content, see "Metabolism of Inorganic Nitrates and Nitrites" in this section.

11. All of the following are true regarding the biological fate of nitrates **EXCEPT**

    A. A majority of ingested nitrate is excreted in the urine within 24 hours.
    B. Reduction of nitrates to nitrites can be beneficial.
    C. Nitrates can be formed endogenously.
    D. The majority of total nitrate intake is converted to nitrite by bacteria in the saliva, stomach, and small intestine.

    To review relevant content, see "Metabolism of Inorganic Nitrates and Nitrites" and "Excretion" in this section.

What Are the Health Effects from Exposure to Nitrates and Nitrites?

Learning Objective

Upon completion of this section, you will be able to

- Describe mechanisms contributing to health effects from exposure to nitrates and nitrites.
- Describe the health effects from exposure to nitrates and nitrites.

Introduction

Unless conditions exist for reducing nitrate to nitrite in the gut (i.e., high pH and proper intestinal microbial flora), ingested nitrate (NO_3^-) is metabolized and excreted without producing apparent adverse effects.

- Nitrate in the diet may even enhance host defenses against gastrointestinal pathogens by modulating platelet activity, and possibly even gastrointestinal motility and microcirculation [McKnight et al. 1999; Lundberg et al. 2004; Hill 1999; Lundberg et al. 2008; Webb et al. 2008; Borniquel et al. 2010; Petersson et al. 2007; 2002; Sobko et al. 2006].
- The known toxic effects of nitrate exposure result from the conversion of nitrate to nitrite [Hord 2011; ATSDR 2004].
- The effects of nitrite (NO_2^-) are the same whether nitrite-containing compounds are ingested or inhaled, or nitrite is produced in vivo from nitrate.

Methemoglobinemia is the critical health effect from exposure to nitrates and nitrites. Depending on the percentage of total MetHb, the clinical presentation may be one of oxygen deprivation with cyanosis, cardiac dysrhythmias and circulatory failure, and progressive central nervous system (CNS) effects [Skold et al. 2011]. CNS effects can range from mild dizziness and lethargy to coma and convulsions [Fan and Steinberg 1996; Bradberry 2003; Osterhoudt 2001; Skold et al. 2011] (See Table 3).

Hematologic Effects

Acute acquired methemoglobinemia is the most important adverse health effect caused by excessive nitrate or nitrite exposure. Methemoglobinemia inducers also work through other mechanisms outside of nitrate and nitrite formation [Nelson and Hostetler 2003; Flomenbaum et al. 2006; Hunter et al. 2011] (See Table 2).

Methemoglobinemia may arise from various etiologies [Harvey et al. 2010; Greer and Shannon 2005; Wright et al. 1999; Nelson and Hostetler 2003]. These etiologies can be grouped into “acquired” and “congenital”. The acquired methemoglobinemias can come from exogenous or endogenous causes.

Exogenous Causes include

- Ingestion, inhalation or dermal exposure to an oxidizing drug or chemical
- Nitrate or nitrite ingestion in water or diet

Endogenous Causes include

- Systemic acidosis as a result of diarrhea and dehydration
- Gastroenteritis without systemic acidosis

Genetic Causes include

- Genetic disorders presenting as cyanosis shortly after birth:
    - NADH methemoglobin reductase deficiency (deficiency of enzymes that reduce MetHb back to Hb)
        - Type 1- RBC reductase deficiency
        - Type 2- Generalized reductase deficiency
    - HbM Disease

“Pseudomethemoglobinemias” may occur from misinterpreted co-oximetry results and include sulfhemoglobinemia [Haymond et al. 2005].

Methemoglobin can be formed by directly oxidizing the iron within the hemoglobin molecule or indirectly causing oxidation through the release of free radicals [Lopez-Shirley et al. 1994; Wright et al. 1999; Nelson and Hostetler 2003; Skold et al. 2011].

Methemoglobinemia is a well-recognized hazard of ingestion of nitrates and nitrites [Hord 2011; Knobeloch et al. 2000; Harris et al. 1979; AAP 1970; Kross et al. 1992].

- The first reported case of fatal acquired methemoglobinemia in an infant due to ingestion of nitrate-contaminated well water in the United States occurred in 1945 [Comly 1945].
- This condition is also termed “Blue Baby Syndrome”.
- In the following 25 years, about 2,000 similar cases of acquired methemoglobinemia in young infants were reported worldwide; about 10% of such cases resulted in death [Reynolds 2002].
- Sporadic cases and occasional fatalities occurred through the 1980s,1990s and 2000s, most often resulting from ingestion of nitrate-contaminated well water by infants [Fan and Steinberg 1996; Knobeloch et al. 2000; Shearer et al. 1972].
- Methemoglobinemia from ingestion of nitrates involves conversion in the intestinal flora of nitrates to nitrites which are absorbed systemically and act as oxidizing agents [Nelson and Hostetler 2003; Skold et al. 2011].

Hemoglobin molecules contain iron within a porphyrin heme structure.

- The iron in hemoglobin is normally found in the Fe^{++} state.
- The iron moiety of hemoglobin can be oxidized to the Fe^{+++} state to form methemoglobin (MetHb).
- Once it is formed, the molecule loses its ability to carry molecular oxygen and reduces its ability to release oxygen to tissues. The increased affinity for bound oxygen results in a left shift of the oxygen-hemoglobin dissociation curve (see Figure 4).

A certain amount of physiologic MetHb formation occurs continuously because red blood cells are bathed in oxygen.

- Several endogenous reduction systems exist to maintain MetHb in the reduced state.
- In normal individuals only about 1% of total hemoglobin is MetHb at any given time [Wright et al. 1999; Jaffe and Hultquist 1995; Skold et al. 2011].

MetHb can be reduced back to hemoglobin by both NADH-dependent and NADPH-dependent (to a lesser degree) MetHb reductase enzymes.

More specifically, the RBC systems responsible for methemoglobin reduction under physiologic conditions include (in order of decreasing methemoglobin reduction):

- NADH methemoglobin reductase (also known as cytochrome b5 methemoglobin reductase, diaphorase I, DPNH-diaphorase, DPNH dehydrogenase I, NADH dehydrogenase, NADH methemoglobin-ferrocyanide reductase)
- Ascorbic acid
- Glutathione
- NADPH methemoglobin reductase (also known as diaphorase II, NADPH dehydrogenase)

[Haymond et al. 2005; McKenzie 2010].

Methemoglobinemia occurs when these systems become overwhelmed, impaired, are lacking or when there is an inherited defect in the structure of the hemoglobin molecule itself (HbM disease) [Nelson and Hostetler 2003; DeBaun et al. 2011; McDonagh et al. 2013].

Two types of inherited enzyme deficiency methemoglobinemia exist. Erythrocyte reductase deficiency occurs when red blood cells lack the enzyme. Generalized reductase deficiency occurs when the enzyme isn't functional anywhere in the body [DeBaun et al. 2011].

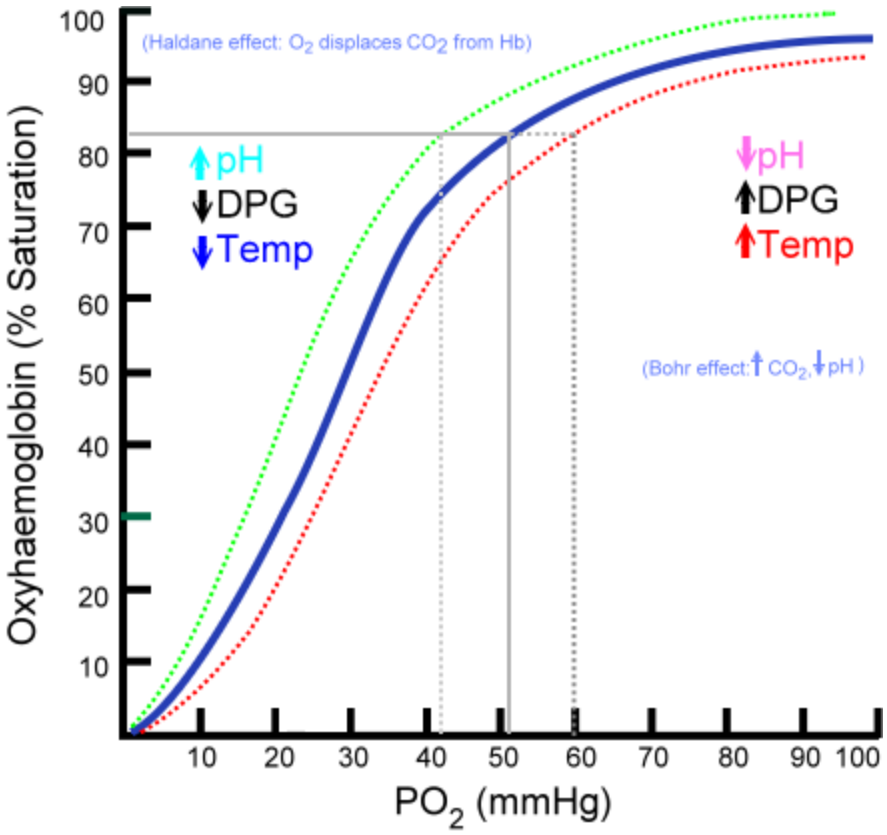


Figure 4. Oxy-Hemoglobin Dissociation Curve. Image Courtesy of Wikimedia Commons viewed in Grethlein SJ and Besa EC. (2012, June 25). Blood Substitutes. Medscape. Retrieved 10/22/13 from http://emedicine.medscape.com/article/207801-overview

Blue line = Normal Hemoglobin
Green line = High affinity Hb, Methemoglobin, “left shift”
Red line = Low affinity Hb, “right shift”

Methemoglobinemia causes a leftward shift in the oxygen-hemoglobin dissociation curve as methemoglobin does not unload O2 from Hb. Sulfhemoglobin causes a right shift in the oxygen-hemoglobin dissociation curve.

Cardiovascular Effects	Hypotension is the main cardiovascular effect seen with nitrate and nitrite medications and previously thought to be uncommon with ingestion of nitrates and nitrites in food and water. However, there have been recent studies looking at potential benefits of dietary inorganic nitrates in promoting cardiovascular health [Lundberg et al. 2011; Larsen et al. 2010; Lauer et al. 2008; Sobko et al. 2010; Vanhatalo et al. 2010; Carlstrom et al. 2011; Casey et al. 2007; Webb et al. 2008]. Angina-like pain, MI and cardiovascular death have been reported in explosive manufacturing industry workers exposed to nitroglycerin and other aliphatic nitrates [Hogstedt and Axelson 1977; Hannerz and Greitz 1992; RuDusky 2001]. In the body, nitroglycerin (similar to other nitrites and organic nitrates) is metabolized to nitric oxide (NO) which stimulates a series of events that eventually results in release of calcium ions from smooth muscle cells leading to relaxation and vasodilation. Increased blood flow in the middle cerebral artery and increased cerebrospinal fluid pressure have been correlated with headache after nitroglycerin exposure [Hannerz and Greitz 1992]. Since the late 1800s, there have been anecdotal reports of explosives workers placing small amounts of explosives in their hatbands when away from work to avoid “powder head” headaches and chest pain on their return to work [Rosenman 2007]. Take home exposures to nitrate dusts on work clothes have been reported to cause headache in exposed family members [Rosenman 2007]. Anecdotal reports of “sudden death” or “Monday morning angina” leading to death were first described in the 1930s as having been associated with dermal absorption of nitroglycerin and ethylene glycol dinitrate, particularly after being away from work/exposure for a short period of time (i.e., a couple of days/over weekends). Rebound coronary

	spasm from withdrawal of nitrates is thought to be the underlying mechanism [RuDusky 2001]. Some studies have shown increases in mortality among occupational cohort's months to years after exposure which would suggest other processes may be involved. Studies and post autopsy reports have supported increased mortality from strokes and heart disease from chronic exposure [Rosenman 2007]. Other studies have not shown increased cardiovascular disease risk from occupational exposure to nitrates [Stayner et al. 1992].
Reproductive and Developmental Effects	Maternal exposure to environmental nitrates and nitrites may increase the risk of pregnancy complications such as • Anemia, • Threatened abortion/premature labor, or • Preeclampsia [Grant et al. 1995; Tabacova et al. 1997]. Recent epidemiologic data have suggested an association between developmental effects in offspring and the maternal ingestion of nitrate from drinking water such as • Spontaneous abortions, • Intrauterine growth restriction, and • Various birth defects. However, a definite conclusion on the cause-and-effect relationship cannot be drawn (i.e. in some studies, the potential for confounding could not be determined with certainty due to lack of individual exposure assessment data, etc.) [Manassaram et al. 2006; Fan and Steinberg 1996; Grant et al. 1995; Huber et al. 2013]. The maternal transfer of nitrate, nitrite, and N-nitroso compounds and the potential effect on fetal death and malformation have been described [Bruning-Fann and Kaneene 1993]. Reproductive outcome studies performed at sites with high nitrate levels in the water supply provide some evidence of maternal transfer of nitrate and nitrite [Manassaram et al. 2006; Tabacova et al. 1997 and 1998; Croen et al. 2001].

	Further study is needed to determine the relationship between maternal exposure to nitrates and nitrites and reproductive and developmental effects.
Other Effects	A few studies have hinted at a role for nitrate intake in the risk for developing diabetes mellitus in childhood [Kostraba et al. 1992; Virtanen et al. 1994; Parslow et al. 1997]. Raynaud phenomena and peripheral neuropathy have been reported in nitrate exposed workers [Rosenman 2007].

Carcinogenicity	Some study results have raised concern about the cancer-causing potential of nitrates and nitrites used as preservatives and color-enhancing agents in meats [Norat et al. 2005; Tricker and Preussmann 1991]. Nitrates can react with amino acids to form nitrosamines, which have been reported to cause cancer in animals [Bruning-Fann and Kaneene 1993]. Elevated risk of non-Hodgkin's lymphoma [Ward et al. 1996] and cancers of the esophagus, nasopharynx, bladder, colon, prostate and thyroid have been reported [Cantor 1997; Eichholzer and Gutzwiller 1998; Barrett et al. 1998; Ward et al. 2010]. An increased incidence of stomach cancer was observed in one group of workers with occupational exposures to nitrate fertilizer; however, the weight of evidence for gastric cancer causation is mixed [Van Loon et al. 1998; Xu et al. 1992]. Epidemiological investigations and human toxicological studies have not shown an unequivocal relationship between nitrate intake and the risk of cancer [Alexander et al. 2010; Mensinga et al. 2003]. The International Agency for Research on Cancer (IARC) classifies nitrates and nitrites as "probably carcinogenic to humans" (Group 2A) under certain conditions (i.e. ingested nitrate or nitrite under conditions that result in endogenous nitrosation) which could lead to the formation of known carcinogens such as N-nitroso compounds [IARC 2010].
Key Points	• Acute acquired methemoglobinemia is the most important adverse health effect caused by excessive nitrate/nitrite exposure. • The known toxic effects from nitrate exposure result from the conversion of nitrate to nitrite. • The effects of nitrite (NO_2^-) are the same whether nitrite-containing compounds are ingested or inhaled, or nitrite is produced in vivo from nitrate. • Maternal exposure to environmental nitrates and nitrites may increase the risk of pregnancy complications such as anemia, threatened abortion/premature labor, or preeclampsia. • Angina-like pain, MI, and cardiovascular death have been reported in explosive industry workers

exposed to nitroglycerin and other aliphatic nitrates.

- Epidemiological investigations and human toxicological studies have not shown an unequivocal relationship between nitrate intake and the risk of cancer.

Progress Checks

12. Effects of methemoglobinemia include which of the following

    A. Cyanosis.
    B. Coma or convulsions.
    C. Dysrhythmias.
    D. All of the above.

    To review relevant content, see "Hematologic Effects" in this section.

13. In methemoglobinemia, the oxidized Fe^{3+} of the hemoglobin molecule

    A. Turns the blood bright red.
    B. Decreases its ability to carry oxygen.
    C. Activates the clotting cascade.
    D. Produces fever.

    To review relevant content, see "Hematologic Effects" in this section.

14. Which of following is/are true regarding health effects from exposure to nitrates and nitrites?

    A. Cardiovascular effects have only been reported with medicinal exposures to nitrates and nitrites.
    B. Maternal exposure to environmental nitrates and nitrites may increase the risk of pregnancy complications.
    C. Nitrates and nitrites are categorized as "carcinogenic to humans" by EPA.
    D. The health effects from nitrite (NO2–) that is produced endogenously from nitrate differ from those caused by ingestion or inhalation of nitrite-containing compounds.

To review relevant content, see "Introduction", "Cardiovascular Effects", "Reproductive and Developmental Effects" and "Carcinogenicity" in this section.

Clinical Assessment - Evaluation

Learning Objectives

Upon completion of this section, you will be able to

- Describe the clinical assessment of an infant with cyanosis due to overexposure to nitrates and nitrites.
- Describe the signs and symptoms of methemoglobinemia.

Introduction

The evaluation of nitrate/nitrite-related health effects most often presents as a clinical evaluation of an infant with cyanosis. Symptomatic methemoglobinemia is generally less common in older children and adults.

While systematically working through the differential diagnoses with special emphasis on airway, pulmonary and circulatory causes, appropriate supportive care tailored to the individual patient's clinical status should be provided.

The differential diagnosis for cyanosis in an infant includes (by mechanism)

- Alveolar Hypoventilation
  - CNS depression (i.e., asphyxia, seizure, meningitis, encephalitis, intraventricular hemorrhage, drug induced)
  - airway obstruction (i.e., choanal atresia, laryngomalacia)

- neuromuscular disease (i.e., phrenic nerve injury, myasthenia gravis)

- Decreased oxygen carrying capacity of blood (less oxygen available at tissue level)
  - Methemoglobinemia (acquired or congenital)

- Decreased peripheral circulation (peripheral cyanosis)
  - Sepsis
  - Shock (any cause)
  - Polycythemia
  - Hypothermia
  - Hypoglycemia
  - Low cardiac output (i.e., hypocalcemia, cardiomyopathies)

- Impaired Oxygen Diffusion
  - Pulmonary edema (i.e. left sided obstructive cardiac disease as seen with aortic stenosis, cardiomyopathy)
  - Pulmonary fibrosis

- Right–to–left shunt
  - Cardiac anomalies (i.e. tetralogy of fallot, transposition of the great arteries, truncus arteriosus, total anomalous pulmonary venous return, tricuspid atresia, pulmonary atresia, hypoplastic left heart)
  - Persistent pulmonary hypertension of the newborn
  - Pulmonary anomalies (i.e., pulmonary arteriovenous malformation)

- Ventilation/perfusion mismatch
  - Airway disease (i.e. transient tachypnea of the newborn (TTN), respiratory distress syndrome (RDS), pneumonia, aspiration, atelectasis, diaphragmatic hernia, pulmonary hypoplasia, pulmonary hemorrhage)

- Extrinsic compression of the lungs (i.e., pneumothorax, pleural effusion, hemothorax)

In an infant with no known cardiopulmonary disease, cyanosis that is unresponsive to oxygen therapy is most likely due to methemoglobinemia. Methemoglobinemia is a condition of excess oxidized “ferric” hemoglobin where the reducing systems to return hemoglobin to a ferrous state are overwhelmed, impaired, or lacking [Nelson and Hostetler 2003].

Causes of methemoglobinemia can be generally grouped into three categories: endogenous (i.e., related to diarrhea, systemic acidosis, infection); exogenous (i.e. medication or toxin-induced); and genetic (i.e., related to methemoglobin reductase enzyme system deficiency or structural variant of hemoglobin (HbM)) [Nelson and Hostetler 2003, Wright et al. 1999].

A high index of suspicion is the key to proper and timely diagnosis. Note that methemoglobinemia can also occur with subtle or no symptoms depending on the methemoglobin level. Methemoglobin occurs when methemoglobin comprises more than 1% of the hemoglobin [Flomenbaum et al. 2006; DeBaun et al. 2011; Fernandez-Frackelton and Bocock 2009; McDonagh et al. 2013]. This can occur when the methemoglobin reductase system is overwhelmed (acquired methemoglobinemia) or deficient (congenital methemoglobinemia) and when a structural hemoglobin variant (hemoglobin M) is present (congenital methemoglobinemia) [DeBaun et al. 2011].

Other forms of abnormal hemoglobin (dyshemoglobin) that also have an impaired ability to transport oxygen and carbon dioxide include carboxyhemoglobin and sulfhemoglobin. Therefore, carboxyhemoglobinemia and sulfhemoglobinemia should also be considered in the differential diagnosis.

	Cyanosis from inherited and acquired forms of methemoglobinemia may present differently depending on the developmental stage of the patient. This is due to developmental differences in amounts of adult (HbA) and fetal hemoglobin (HbF) present as well as the presence of other structural variants of hemoglobin [McKenzie 2010]. Infants with inherited methemoglobin reductase enzyme deficiencies may present with cyanosis and elevated levels of MetHb shortly after birth [DeBaun et al. 2011]. There are several variants of hereditary hemoglobin M (HbM). Alpha chain variants may present with cyanosis at birth, whereas those with beta chain variants may not show cyanosis until 4-6 months after birth [DeBaun et al. 2011]. Consideration should also be given to the fact that carbon monoxide poisoning doesn't produce cyanosis [Haymond et al. 2005].
Exposure History	The evaluation of a patient with suspected nitrate or nitrite exposure includes a complete medical and exposure history [ATSDR 2008]. For information on taking a complete exposure history including questions to ask adults and children, see ATSDR Case Studies in Environmental Medicine: Taking an Exposure History https://www.atsdr.cdc.gov/csem/csem.asp?csem=33&po=0 and ATSDR Case Studies in Environmental Medicine: Taking a Pediatric Exposure History http://www.atsdr.cdc.gov/csem/csem.asp?csem=26&po=0

	Clues to potential nitrate or nitrite exposure are often obtained by questioning the patient or family about the following subset of topics • Location of the home (urban, suburban, or rural), • Drinking water source and supply (if well water: o Depth, o Location, o Type of well construction, and o Frequency of microbiologic and nitrate testing), • Nearby activities (agricultural or industrial) and proximity to drinking-water source, • Type of sewer system (municipal or septic) and proximity to drinking-water source, • Proximity of neighboring septic tanks or others up gradient to drinking water source, • Recent flooding, • Occupations, avocations, and hobbies of family members, • Type of formula consumed by infant, feeding regimen, and source of dilution water, • Types of food eaten, with a focus on prepared meats, carrots, spinach, and beets, • Recent use of medications by infant and mother. See Table 2 for a select list of methemoglobin (MetHb) inducers.
Medical History	Additional questions should be asked about the medical history including • Family history, • Known blood or enzyme disorders, • Nutritional status and growth history, • History of recent gastroenteritis with vomiting or diarrhea, • Other episodes of cyanosis, recently or as a newborn, and • History of tachypnea, tachycardia, or hypotension.
Physical Examination	All cyanotic patients should be assessed for possible cardiac and lung disease including • Cardiac murmurs,

- Gallops,
- Arrhythmias,
- Rales,
- Rhonchi,
- Wheezes,
- Dullness, or
- Hyperresonance in the chest.

A central chocolate-brown or slate-gray cyanosis that does not respond to administration of 100% oxygen is suggestive of methemoglobinemia [Wright et al. 1999; Rehman 2001; Bradberry 2003; Denshaw-Burke 2013; Steinhorn 2008; Skold et al. 2011]. In addition, the patient is often (but not always) less ill than one would expect from the severity of cyanosis [Bradberry 2003; Denshaw-Burke 2013]. On the other hand, infants with a large proportion of fetal hemoglobin may have severely reduced oxygenation before cyanosis appears clinically [Steinhorn 2008].

Physical examination should include special attention to the color of the skin and mucous membranes. In young infants, look for

- Labored breathing,
- Respiratory exhaustion,
- Hypotension,
- Below-average weight gain, and
- Failure to meet developmental indices.

Note that for best results, the physical exam should be performed on an appropriately warmed and calmed infant [Steinhorn 2008].

Gastroenteritis can increase the rates of production and absorption of nitrites in young infants and cause or aggravate methemoglobinemia [Nelson and Hostetler 2003]. If gastroenteritis is present—especially in infants—evaluate the patient for the possible presence of dehydration (i.e., poor skin turgor, sunken fontanel, dry mucous membranes) [Wright et al. 1999; Zorc and Kanic 2001; Nelson and Hostetler 2003].

Fetal Hemoglobin (HbF)

Fetal hemoglobin (HbF) is structurally different than normal adult hemoglobin (HbA). HbF is made up of two gamma and two alpha chains while adult hemoglobin (HbA) is made of two alpha and two beta chains. The level of fetal hemoglobin varies by developmental stage. The reference intervals by developmental stage include

> HbF: 90-95% before birth, 50-85% at birth, <2% at > 1 year to adult
>
> HbA: 10-40% at birth, > 95% at > 1 year to adult

[McKenzie 2010].

The proportions of each hemoglobin will affect the oxygen saturation at a given PaO2. For example, if an infant has mostly adult hemoglobin and the PaO2 drops below 50 mmHg, a central cyanosis (arterial saturation 75-85%) can appear. If the infant has mostly fetal hemoglobin, a clinical cyanosis may not appear until the PaO2 drops below 40 mmHg. Therefore, infants with mostly fetal hemoglobin can have severe reductions in oxygenation before cyanosis appears clinically [Steinhorn 2008]. (See Figure 5.)

In addition, because most clinically relevant hemoglobinopathies involve beta chain alterations, having higher HbF levels will affect the timing of the clinical appearance of these conditions.

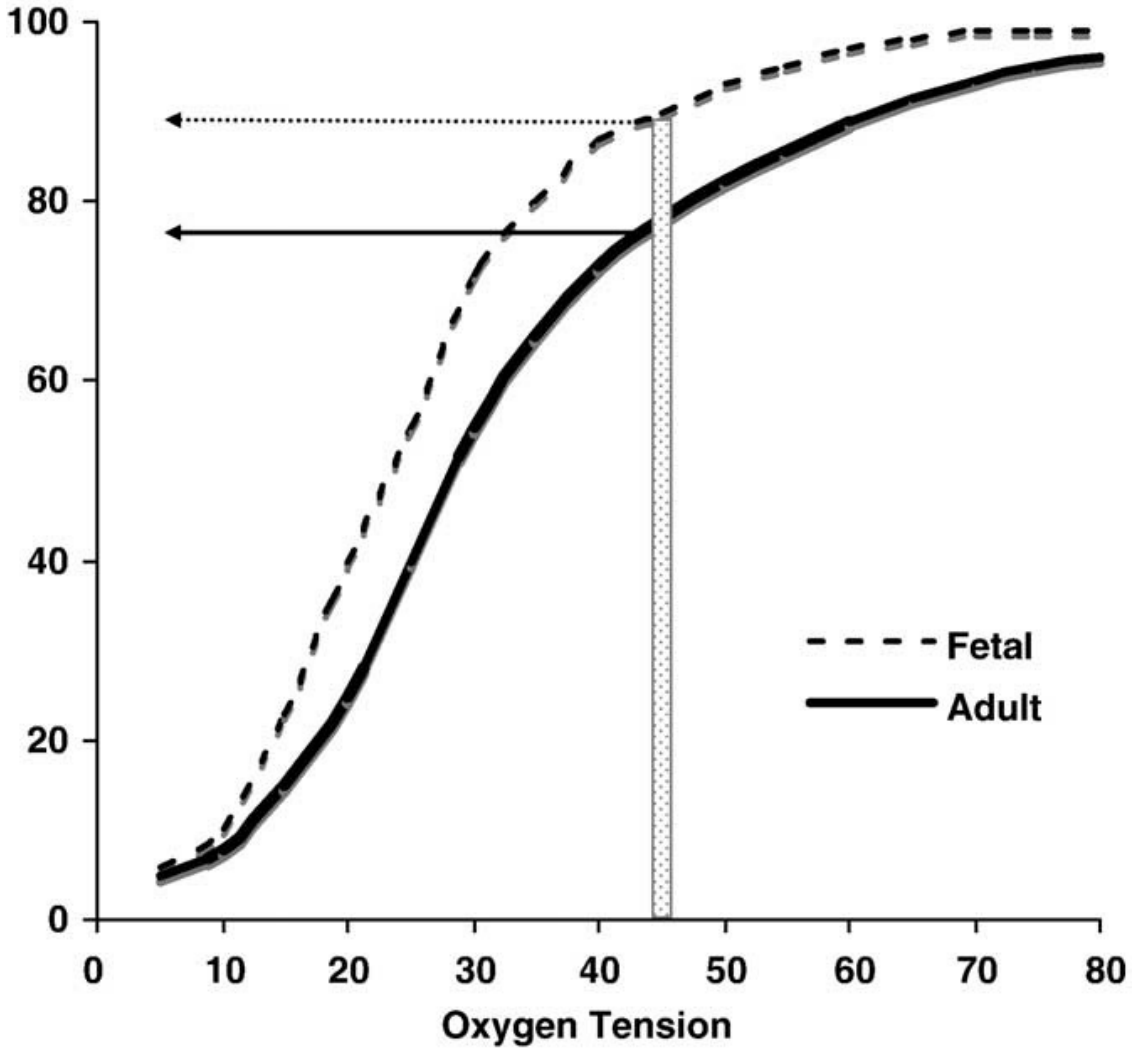


Figure 5. Representation of the different characteristics of oxygen binding in fetal vs. adult hemoglobin. The structural differences between fetal hemoglobin (HbF) and normal adult hemoglobin (HbA) result in HbF's leftward shift from the HbA dissociation curve. HbF has a higher affinity to bind oxygen at lower partial pressures. The transition from predominately HbF to predominately HbA varies by developmental stage. For example, at a PaO2 of 45 mmHg, an infant with more HbF than HbA may not show clinical cyanosis (typically seen at about 80% oxygen saturation) as would an adult or infant with higher amounts of HbA.

Figure adapted from Steinhorn 2008. (Open access, public domain author manuscript in NIH public access PMC system).

Table 2. Reported Inducers of Methemoglobinemia

Agent	**Source / Use**
Inorganic nitrates/nitrites	• Contaminants of nitrous oxide canisters for anesthesia • Contaminated well water • Industrial salts • Meat preservatives • Silver nitrate burn therapy • Vegetables: carrot juice, spinach, beets • Fava beans (esp. ingestion by G6PD deficient patients) • Instant cold packs • Agricultural fertilizers • Oxidizing agents in explosives (such as ammonium nitrate) • Oxidizing agents in production of methamphetamine (such as ammonium nitrate)
Organic nitrates	
• Butyl/isobutyl nitrate • Amyl/sodium nitrate • Nitroglycerine	• Room deodorizer propellants • Inhalant in cyanide antidote kit • Inhalation abuse “poppers” • Oral, sublingual, or transdermal pharmaceuticals for treatment of angina
Others	
• Acetaminophen • Aniline Dyes • Aminophenols • Herbal Medicines	• Laundry ink, colored wax crayons (esp. red), diaper marking ink, freshly dyed shoes or blankets • Ginkgo biloba (Chinese herbal medicine, high dose adverse effect)

• Nitrobenzene	• Industrial solvents • Gun-cleaning products
• Nitroethane	• Found in nail polish, resins, rubber adhesives
• Local anesthetics	• Benzocaine (used as spray: endotracheal intubation, transesophageal echocardiography (TEE), esophagogastroduodenoscopy (EGD), bronchoscopy; as a topical cream for hemorrhoids or teething preparation, as an adulterant in cocaine, recreational drugs) • Lidocaine • Propitocaine • Prilocaine • EMLA (Eutectic Mixture of Local Anesthetics) topical anesthetic (lidocaine 2.5% and prilocaine 2.5%)
• Sulfonamides	• Antibacterial drugs*
• Phenazopyridine	• Pyridium
• Antimalarials	• Chloroquine, • Primaquine
• Sulfones	• Dapsone*
• *p*-Aminosalicylic acid	• Bactericide (tuberculostatic)
• Naphthalene	• Mothballs
• Copper sulfate	• Fungicide for plants • Seed treatments
• Resorcinol	• Antiseborrheic • Antipruritic • Antiseptic
• Chlorates	• Matches

	• Explosives • Pyrotechnics
• Combustion products	• Fires • Automobile exhaust • Fume from burning plastics and wood
• Herbicides and Pesticides • Petrol octane booster	
• Other medications	• Acetanilide • Chloramine • Flutamide • Metochlopramide • Nitric Oxide (inhaled) • Nitrofurans • Nitroprusside • Paraquat • Phenacetin • Zopiclone
Medical conditions	• Pediatric gastrointestinal infection • Sepsis • Sickle Cell Crisis

*Can also induce sulfhemoglobinemia
Adapted from [Dabney et al. 1990]; Updated using [Ash-Bernal et al. 2004; Skold et al. 2011; Hunter et al. 2011]

Correlation of Signs and Symptoms with MetHb Levels	The signs and symptoms of methemoglobinemia listed in Table 3 can be roughly correlated with the percentage of total hemoglobin in the oxidized form (see “Clinical Assessment- Laboratory Tests”). Unfortunately, because methemoglobin (MetHb) is generally expressed as a percent of total hemoglobin, levels may not correspond with symptoms in some patients. For example, a patient with a MetHb level of 20% and total hemoglobin of 15 grams per deciliter (g/dL) still has 12 g/dL of functioning hemoglobin, whereas a patient with a MetHb level of 20% and total hemoglobin of 8 g/dL has only 6.4 g/dL of functioning hemoglobin. Anemia, acidosis, respiratory compromise, cardiovascular disease, sepsis or the presence of other abnormal hemoglobin species (i.e. carboxyhemoglobin, sulfhemoglobin, sickle hemoglobin (HbS) may make patients more symptomatic than expected for a given MetHb level [Wright et al. 1999; Ash-Bernal et al. 2004; Skold et al. 2011]. Due to the large excess capacity of the blood to carry oxygen, levels of MetHb up to 10% typically do not cause significant clinical signs in an otherwise healthy individual. Levels above 10% may result in cyanosis, weakness, and rapid pulse [Ash-Bernal et al. 2004; Hunter et al. 2011]. Patients with comorbidities that decrease oxygen transport or delivery may develop moderate to severe symptoms at much lower MetHb levels than a previously healthy patient [Hunter et al. 2011]. A chocolate-brown or slate-gray central cyanosis—involving the trunk and proximal portions of the limbs, as well as the distal extremities, mucous membranes, and lips—is one of the hallmarks of methemoglobinemia and can become noticeable at a concentration of 10%–15% of total hemoglobin [Mack 1982; Geffner et al. 1981; Wentworth et al. 1999; Skold et al. 2011]. Dyspnea and nausea occur at MetHb levels of above 30%, while lethargy and decreased consciousness occur as levels approach 55%. Higher levels may cause cardiac arrhythmias, circulatory failure, and neurological depression. Levels above 70% are often fatal [Coleman and Coleman 1996; Hunter et al. 2011; Skold et al. 2011]. Features of toxicity may develop over hours or even days [Bradberry 2003; Hunter et al. 2011].

Table 3. Signs and Symptoms of Methemoglobinemia

Methemoglobin Concentration (%)	Clinical Findings
10–20	• Central cyanosis of limbs/trunk; often asymptomatic but may have weakness, tachycardia
20–35	• Central nervous system depression (headache, dizziness, fatigue), • Dyspnea, • Nausea
35–55	• Lethargy, • Syncope, • Coma, • Arrhythmias, • Shock, and • Convulsions.
>70	• High risk of mortality

Adapted from [Dabney et al 1990]; Updated from [Ash-Bernal 2004; Hunter et al. 2011; Skold et al. 2011].

Key Points

- The evaluation of nitrate/nitrite-related health effects most often presents as a clinical evaluation of an infant with cyanosis.
- A central chocolate-brown or slate–gray central cyanosis that does not respond to administration of 100% oxygen is suggestive of methemoglobinemia.
- A patient with methemoglobinemia may appear less ill than what would be expected from the severity of cyanosis, but not always.
- Infants with a large proportion of HbF may have severely reduced oxygenation before cyanosis appears clinically.
- Exposure history for infants should focus on formula preparation and the source of formula dilution water.
- Signs and symptoms of methemoglobinemia are roughly correlated with the percentage of oxidized hemoglobin in the blood.
- Patients with comorbid health conditions that impair oxygen transport may have symptoms at lower MetHb levels than an otherwise healthy patient.

- Symptoms of MetHb can be ambiguous and nonspecific. Therefore, a high index of suspicion for MetHb is imperative for early diagnosis and treatment.

Progress Checks

15. What key areas should be addressed in the exposure history?

    A. Recent use of medications by infant and mother.
    B. Type of formula, feeding regimen, and source of dilution water.
    C. Drinking water source and supply.
    D. All of the above.

    To review relevant content, see "Exposure History" in this section and Table 2.

16. What level of MetHb creates a high mortality risk in an otherwise healthy individual?

    A. 20%.
    B. 40%.
    C. 70%.
    D. None of the above.

    To review relevant content, see "Correlation of Signs and Symptoms with MetHb Levels" in this section and Table 3.

17. Which of the following is/are true regarding the clinical assessment?

    A. All cyanotic patients should be assessed for possible cardiac and lung disease (cardiac murmurs, gallops, arrhythmias, rales, rhonchi, wheezes, dullness, or hyperresonance in the chest).
    B. A central chocolate-brown or slate-gray cyanosis that does not respond to administration of 100% oxygen is suggestive of methemoglobinemia.
    C. The victim is often less ill than one would expect from the severity of 'cyanosis'.
    D. All of the above.

To review relevant content, see "Physical Examination" in this section.

Clinical Assessment – Laboratory Tests

Learning Objectives	Upon completion of this section, you will be able to • Identify the laboratory test results that indicate methemoglobinemia.
Introduction	The evaluation of cyanosis in an infant should systematically work through the differential diagnoses with special emphasis on airway, pulmonary and circulatory causes. In cases of severe cyanosis, urgent supportive therapy (i.e. intravenous fluids, "thermoneutral" environment, glucose infusions/monitoring, airway or assisted ventilation depending on clinical presentation of patient/level of respiratory distress, etc.) should be provided while a diagnosis is established [Steinhorn 2008]. A typical cyanosis workup includes CBC with differential and peripheral blood smear, free serum hemoglobin and haptoglobin, ABGs and pulse oximetry. Imaging (chest x-ray) and/or functional studies (echocardiogram, EKG) to assess cardiac and/or pulmonary status may be necessary based on clinical presentation. Increased suspicion for methemoglobinemia is central to timely and accurate diagnosis. Methemoglobin results in distinct changes in blood color and oxygen-carrying capacity. Methemoglobinemia can be acquired (exposure to oxidants) or inherited (i.e. decreased enzyme activity or presence of hemoglobin M). Acquired

	methemoglobinemia will have normal enzyme assay activity tests and normal Hb electrophoresis. For hereditary methemoglobinemias, reduced enzyme activity is seen with NADH-methemoglobin reductase deficiency, but normal in HbM disease. Hemoglobin electrophoresis is abnormal in HbM disease, but normal with NADH-methemoglobin reductase deficiency [McKenzie 2010]. Table 4 summarizes suggested lab tests for a methemoglobinemia work up.
Screening Tests Included in a Methemoglobin-emia Work Up	A typical methemoglobin work up includes [Denshaw-Burke 2013]. Tests to rule out hemolysis include CBC with differential, reticulocyte count, peripheral blood smear, lactate dehydrogenase (LDH), bilirubin, serum haptoglobin, free serum hemoglobin and Heinz body preparation. The CBC with differential, the RDW, erythrocyte indices and peripheral blood smear can help identify and characterize anemias, distinguish thalassemias from hemoglobinopathies and detect other abnormalities related to the differential diagnoses for cyanosis. Free serum hemoglobin and haptoglobin levels are drawn to assess for hemolytic anemias. A decrease in haptoglobin can support a diagnosis of hemolytic anemia when seen with an increased reticulocyte count, decreased erythrocyte count, decreased hemoglobin and hematocrit. Tests to determine end-organ dysfunction or failure may include liver function tests, electrolytes, renal function tests. Tests to determine functional or structural abnormalities may include imaging studies of the chest, EKG and echocardiogram. Testing should include a urine pregnancy test for females of childbearing age to guide treatment and management decisions.

	Tests to determine oxygen saturation may include (depending on availability) ABG and standard pulse oximetry (a “saturation gap” or difference between the oxygen saturation results of ABG alone (calculated) vs. standard pulse oximetry will be present in methemoglobinemia), ABG with co-oximetry, or multiple wavelength pulse oximetry (also called continuous pulse co-oximetry).
Bedside Testing for MetHb	A screening test for methemoglobinemia that can be done at the bedside is described below: • Place 1 or 2 drops of the patient’s blood on white filter paper. • The chocolate-brown appearance of methemoglobin (MetHb) does not change with time. • In contrast, deoxyhemoglobin appears dark red/violet initially and then brightens after exposure to atmospheric oxygen. • Gently blowing supplemental oxygen onto the filter paper hastens the reaction with deoxyhemoglobin, but does not affect MetHb [Wright et al. 1999; Wentworth et al. 1999; Haymond et al. 2005; Skold et al. 2011; Denshaw-Burke 2013]. • A tube of MetHb-containing blood will not turn red when shaken in air or when oxygen is bubbled through it, whereas blood that is dark because of normal deoxyhemoglobin will turn red [Henretig et al. 1988; Haymond et al. 2005; Skold et al. 2011; Ritchey et al. 2012; Denshaw-Burke 2013].
Standard Pulse-oximetry and Arterial Blood Gases	Standard Pulse-oximetry measurement of the oxygen saturation of hemoglobin does not provide accurate results in the presence of methemoglobinemia [Ralston et al. 1991; Flomenbaum et al. 2006; DeBaun et al. 2011; Skold et al. 2011; Denshaw-Burke 2013]. • Standard pulse oximetry underestimates oxygen saturation at low levels of methemoglobinemia and overestimates oxygen saturation when methemoglobinemia is severe (i.e. lower and higher MetHb levels will show a constant oxygen saturation close to 85%). • Arterial blood gas analysis will typically reveal a normal arterial oxygen tension (PO2) and may

	reveal a metabolic acidosis proportional to the severity and duration of tissue hypoxia. • ABGs indicate plasma oxygen content and therefore don't correspond to the oxygen-carrying capacity of hemoglobin. • The profound and disproportionate metabolic acidosis seen in young infants with diarrheal illness and methemoglobinemia suggests that the acidosis is a cause or coexisting finding rather than a result of methemoglobinemia [Bradberry 2003; Avner et al. 1990; Nelson and Hostetler 2003; DeBaun 2011].
Co-Oximetry and MetHb Levels	MetHb percentages can only be used to estimate oxygen-carrying capacity when interpreted with the total hemoglobin [Osterhoudt 2001; Skold et al. 2011; DeBaun et al. 2011; Denshaw-Burke 2013]. • Many hospital laboratories do not measure oxygen saturation directly on blood gas analysis. Instead, they derive it from a nomogram that is based on the measured PO2 and the presence of normal hemoglobin. In this case, since standard pulse oximetry assumes and is limited to the absorbance characteristics for oxy and deoxyhemoglobin, the calculated oxygen saturation could be falsely elevated in the presence of methemoglobinemia (depending on amount of MetHb present; doesn't distinguish between the overlapping absorbance characteristics of MetHb). • A "saturation gap" exists when the measured oxygen saturation of blood differs from the oxygen saturation calculated by routine blood gas analysis. • A saturation gap of more than 5% suggests the presence of MetHb, carboxyhemoglobin, or sulfhemoglobin [Coleman and Coleman 1996; Park and Nagel 1984; Skold et al. 2011; Flomenbaum et al. 2006; DeBaun et al. 2011]. Co-oximetry is an accurate method of measuring MetHb [Skold et al. 2011; Denshaw-Burke 2013]. • A co-oximeter is a simplified spectrophotometer, but unlike a standard pulse oximeter that only measures absorbance at two wavelengths, it

	measures light absorbance at multiple different wavelengths to accurately measure the total amount of hemoglobin. • These wavelengths correspond to specific absorbance characteristics including o Deoxyhemoglobin (reduced hemoglobin), o Oxyhemoglobin, o Carboxyhemoglobin, o Methemoglobin, and o Hemoglobin. • Interpreting the results from a blood gas analyzer without co-oximetry may lead to misdiagnosis because the oxygen saturation will have been calculated but not measured [Matthews 1995; Mansouri and Lurie 1993; Skold et al. 2011]. • Sulfhemoglobin and methemoglobin have similar wavelengths which should be considered when interpreting co-oximetry readings. A "pseudomethemoglobinemia" occurs when sulfhemoglobin is erroneously detected as methemoglobin which results in a falsely elevated MetHb level. • Note that lipemic blood specimens may also result in a falsely elevated methemoglobin level.
Pulse Co-Oximetry	Multiple wavelength pulse "co-oximeters" now exist and provide a noninvasive and continuous way to measure MetHb levels and oxygen saturation. Some models can distinguish sulfhemoglobin from methemoglobin [Steinhorn 2008; Macknet et al. 2007; Macknet et al. 2010].
Cyanide Test	This test can both quantify MetHb level and distinguish between sulfhemoglobin and MetHb. Cyanide binds to the positively charged MetHb. This binding eliminates the MetHb light absorption wavelengths in direct proportion to the MetHb concentration. MetHb is given as a percentage of total concentration of hemoglobin [Evelyn and Malloy 1938; Skold et al. 2011]. • Methemoglobin reacts with cyanide to form cyanomethemoglobin which has a bright red color.

Sulfhemoglobin doesn't react with cyanide to create this bright red color [Evelyn and Malloy 1938; Skold et al. 2011; Denshaw-Burke 2013].

- Since methemoglobin has increased red blood cell affinity for cyanide, it can be used in the treatment of cyanide poisoning. Nitrites can be used to oxidize hemoglobin to methemoglobin which can then bind cyanide.

Table 4. Suggested Lab Tests for Methemoglobinemia Work Up

Test	Purpose
Screening Tests	• Examination of blood color (will stay brown in presence of oxygen) • Determination of MetHb level • Determination of the calculated versus measured arterial saturation gap using co-oximetry • Tests to rule out hemolysis and/or characterize any identified anemia (Complete blood count (CBC) with differential, reticulocyte count, peripheral blood smear including Heinz body preparation, lactate dehydrogenase (LDH), bilirubin, serum free hemoglobin and serum haptoglobin. • Tests to determine end-organ dysfunction or failure (liver function tests, electrolytes, renal function tests) • Urine pregnancy tests for females of childbearing age (to guide treatment and management decisions) • Urinalysis (for hemolysis detection- reddish brown color) • If available, ABG with co-oximetry or multiple wavelength pulse oximetry • Potassium cyanide test (can distinguish methemoglobin from sulfhemoglobin) • Diagnostic imaging to exclude pulmonary or cardiac disease/abnormalities o Imaging studies of chest o Echocardiography o EKG

Specialized Tests to Assess congenital Methemoglo-binemia	Tests for causes of congenital methemoglobinemia (i.e., deficiencies of MetHb reducing enzymes or hemoglobin M) include • Hemoglobin electrophoresis and DNA sequencing of globin chain gene (to detect hemoglobin M (HbM) variants which have mutations in the globin chain that stabilize heme iron in the ferric state and may give rise to misleading co-oximetry results) • Specific enzyme assays (often in several cell lines) o Activity of NADH-dependent MetHb reductase (also called cytochrome b5 reductase) o Activity of glucose-6-phosphate dehydrogenase (G6PD) o Activity of NADPH-dependent MetHb reductase
Direct Biologic Indicators	In general, measurements of nitrates or nitrites in blood, urine, or saliva are not clinically useful.
Indirect Biologic Indicators	The most useful diagnostic test for nitrate toxicity is a blood MetHb level. This can accurately be determined using ABGs with co-oximetry (standard co-oximetry can differentiate MetHb from carboxyhemoglobin, oxyhemoglobin and deoxyhemoglobin; newer generation co-oximeters expand this detection capability). Likewise, multiple wavelength pulse-oximeters exist that can noninvasively and continuously determine MetHb levels. Some newer generation multiple wavelength pulse oximeters can distinguish sulfhemoglobin from methemoglobin.

Key Points

- Methemoglobinemia results in distinct changes in blood color and oxygen-carrying capacity.
- Standard pulse-oximetry measurement of the oxygen saturation of hemoglobin does not provide accurate results in the setting of methemoglobinemia.
- Oxygen saturation values from ABG analysis

	(without co-oximetry) is calculated based on a normal hemoglobin nomogram, rather than measured directly. • A “saturation gap” between the measured oxygen saturation of blood (standard pulse oximetry) and oxygen saturation calculated by routine blood gas analysis increases suspicion for methemoglobinemia. • Co-oximetry with ABGs is an accurate method of measuring MetHb levels and oxygen saturation. • Multiple wavelength pulse oximeters exist that can noninvasively measure and continuously monitor MetHb levels and oxygen saturation.

Progress Checks

18. A drop of blood with MetHb appears as what color on filter paper?

    A. Chocolate-brown.
    B. Red.
    C. Violet.
    D. Clear yellow.

    To review relevant content, see "Bedside Testing Instructions" in this section.

19. Of the following, which is the best method for measuring MetHb levels?

    A. Standard ABGs.
    B. ABGs with co-oximetry.
    C. CBC with differential and blood smear.
    D. Standard pulse oximetry.

    To review relevant content, see "Standard Pulse-oximetry and Arterial Blood Gases" and "Co-Oximetry and MetHb Levels" in this section.

20. Clinically useful diagnostic test(s) for nitrate toxicity include

    A. Measurements of **nitrates** in blood, urine, or saliva.
    B. Measurements of **nitrites** in blood, urine, or saliva.
    C. Blood MetHb level.
    D. All of the above.

    To review relevant content, see Table 4.

How Should Patients Overexposed to Nitrates and Nitrites Be Treated and Managed?

Learning Objectives

Upon completion of this section, you will be able to

- Describe treatment modalities in the management of methemoglobinemia caused by nitrate and nitrite toxicity.

Introduction

General principles of supportive care, with attention to removal of the cause, will suffice for most identified cases of methemoglobinemia resulting from nitrates and nitrites. Not all patients require specific antidotal therapy.

For infants, well water used in preparing formula is a primary etiologic suspect. Patients with chronic congenital methemoglobinemia may have adapted to the chronic cyanosis, such that very high levels of methemoglobin (MetHb) are tolerated without any overt symptoms [Wright et al. 1999; Skold et al. 2011]. Proper fluid, electrolyte, and pH balance is vital, especially in infant methemoglobinemia complicated or caused by serious illness [Olsen and McEvoy 1981; DeBaun 2011; Nelson and Hostetler 2003].

To rule out other etiologies, comatose patients may require intravenous naloxone and glucose. Activated charcoal may be administered after ingestion of substances (generally medications or mothballs) known to cause methemoglobinemia (see Table 2) [Lu et al. 1998; McGoldrick and Bailie 1995; Reigart et al. 1982; Bucaretchi et al. 2000; Sillery et al. 2009; Rahman et al. 2012]. For further guidance on activated charcoal use, please consult your poison control center (1-800-222-1222).

Treatment decisions must be made immediately once methemoglobinemia is recognized and confirmed.

Patients who are symptomatic or have significant concurrent problems that compromise oxygen delivery such as

- Heart disease,
- Lung disease,
- Carbon monoxide poisoning, or
- Anemia

	may need antidotal treatment at MetHb levels as low as 10% [Skold et al. 2011]. Because MetHb levels are typically reported as a percentage of hemoglobin, symptoms may vary depending on the total hemoglobin level. As an easy to remember guideline, the treatment action level is often considered to be 20% MetHb in symptomatic patients and 30% in asymptomatic patients [Skold et al. 2011; Osterhoudt 2001; Price 1998]. Monitoring of clinical and laboratory parameters for evidence of escalating or rebound methemoglobinemia, worsening oxygen delivery, or possible hemolysis should be performed during treatment [Bradberry 2003; Osterhoudt 2001; Skold et al. 2011].
Methylene Blue	Methylene blue is an effective antidote for most patients with methemoglobinemia [Skold et al. 2011; McDonagh et al. 2013]. • Methylene blue is provided as a 1% solution (10 milligrams per milliliter (mg/mL). • The dose is 2 milligrams per kilogram body weight (mg/kg) (0.2 milliliters per kilogram (mL/kg) of a 1% solution) infused intravenously over 3 to 5 minutes. • The dose may be repeated at 1 mg/kg if MetHb does not resolve within 30 minutes. Methylene blue should reduce MetHb levels significantly in less than an hour. It does this by acting as a cofactor to increase the activity of NADPH- MetHb reductase [Skold et al. 2011; McDonagh et al. 2013] (See Figure 6). • Under usual conditions, NADPH-dependent MetHb reductase reduces less than 5% of MetHb to Hb (as the NADH-dependent MetHb reductase is the dominate pathway). However, NADPH-MetHb reductase assumes a major role in the pharmacotherapy of methemoglobinemia with administration of methylene blue [Skold et al. 2011; McDonagh et al. 2013].

Infants with methemoglobinemia resulting from diarrhea and acidosis may improve with aggressive hydration and bicarbonate infusion to correct the acidosis. However, MetHb levels greater than 20% in symptomatic patients should be treated with methylene blue [Wright et al. 1999; Nelson and Hostetler 2003; Skold et al. 2011]. A second dose of methylene blue will be required in only very severe cases or if there is evidence of ongoing MetHb formation [Bradberry 2003; Skold et al. 2011; McDonagh et al. 2013]. The total dose should not exceed 7 mg/kg because the drug by itself is an oxidizing agent [Harvey and Keitt 1983; Skold et al. 2011]. Certain drugs, such as dapsone, create MetHb over a prolonged biologic half-life because of ongoing formation of metabolites. In these situations, some clinicians prefer continuous infusions of methylene blue titrated from a starting rate of 0.1 mg/kg/hour, rather than intermittent bolus therapy [Berlin et al. 1985; Prasad et al. 2008; Skold et al. 2011]. Of note is that Dapsone and sulfonamides may also induce sulfhemoglobinemia which is irreversible lasting the lifetime of the red blood cell with no known antidote [Schmitter 1975; Park and Nagel 1984; Turner et al. 2007; Ashurst et al. 2010; Skold et al. 2011; Denshaw-Burke 2013]. Since sulfhemoglobin molecules do not carry oxygen and have a similar wavelength as MetHb, they can result in a misleading co-oximetry interpretation [Nelson and Hostetler 2003; Denshaw-Burke 2013].

Methylene blue may discolor skin and mucous membranes, making visual interpretation of cyanosis inaccurate. It may also interfere further with standard pulse oximetry readings. After administration of methylene blue, it is prudent to reassess the patient's clinical status and current MetHb levels before proceeding with repeat doses [Osterhoudt 2001; Skold et al. 2011]. Methylene blue is excreted primarily by the kidneys. Although side effects are uncommon, large rapidly administered doses have been associated with

- Nausea,
- Retrosternal chest pain,
- Tachycardia,
- Hypertension, and

- Anxiety.

Urine will subsequently develop a blue-green discoloration [Goluboff and Wheaton 1961; Skold et al. 2011].

Because glucose is necessary for the effectiveness of methylene blue, normoglycemic patients should receive maintenance amounts of dextrose and hypoglycemic patients should receive standard dextrose therapy to correct hypoglycemia [Wright et al. 1999; McDonagh et al. 2013].

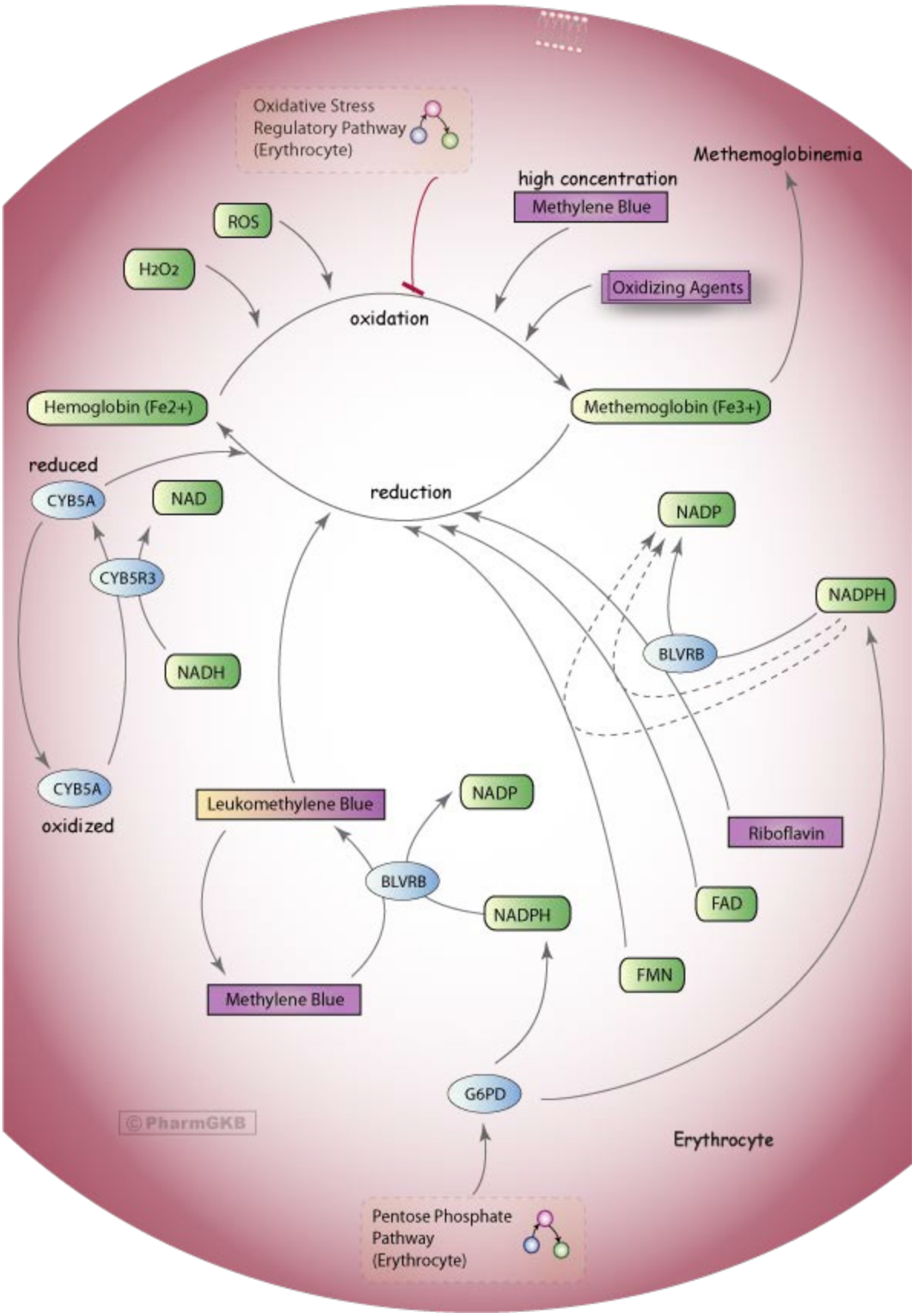

Oxidative Stress
Regulatory Pathway
(Erythrocyte)
Methemoglobinemia
high concentration
Methylene Blue
ROS
H2O2
Oxidizing Agents
oxidation
Hemoglobin (Fe2+)
Methemoglobin (Fe3+)
reduced
CYB5A
NAD
reduction
NADP
CYB5R3
NADPH
BLVRB
NADH
CYB5A
oxidized
Leukomethylene Blue
NADP
Riboflavin
BLVRB
NADPH
FAD
FMN
Methylene Blue
G6PD
©PharmGKB
Erythrocyte
Pentose Phosphate
Pathway
(Erythrocyte)

Figure 6. Methylene Blue Pathway, Pharmacodynamics

A stylized diagram showing the mechanisms that can cause methemoglobin production in erythrocytes and the control mechanisms to prevent methemoglobinemia, including methylene blue treatment which requires NADPH from the Pentose Phosphate Pathway.

http://www.pharmgkb.org/pathway/PA165980834 [McDonagh et al 2013]

Glucose-6 - Phosphate Dehydrogenase (G6PD) Deficiency	Glucose-6-phosphate dehydrogenase (G6PD) is estimated to exist in almost 330 million people around the world, with highest prevalence in Africa, the Middle East and Asia [McDonagh et al. 2013]. Known or suspected G6PD deficiency is a relative contraindication to the use of methylene blue [Chan 1996; Wright et al. 1999; Skold et al. 2011; McDonagh et al. 2013]. • G6PD is a key enzyme in the formation of NADPH, • G6PD-deficient individuals generate insufficient NADPH to efficiently reduce methylene blue to leukomethylene blue, which is necessary for the activation of the NADPH-dependent MetHb reductase system. • G6PD-deficient individuals are also prone to methylene blue-induced hemolysis. • Methylene blue may also add to oxidative hemolysis. However, many G6PD-deficient patients have only a partial enzyme deficiency. In these patients, methylene blue may still lower MetHb levels, and the resultant hemolysis may be mild. Therefore, methylene blue is still the first-line treatment in G6PD-deficient patients with *life-threatening* MetHb [Skold et al. 2011; McDonagh et al. 2013]. A lower starting dose of methylene blue (0.3 to 0.5 mg/kg) is recommended. The dose may be titrated upward to reduce MetHb, as necessary. If the patient's condition worsens, methylene blue treatment should be stopped and exchange transfusion considered [Wright et al. 1999; Skold et al. 2011; McDonagh et al. 2013].

	Young infants without G6PD deficiency have developed Heinz body hemolytic anemia at doses as low as 4 mg/kg [Kirsch and Cohen 1980; Rosen et al. 1971; Nelson and Hostetler 2003]. Moreover, in the presence of hemolysis, high dose methylene blue can itself initiate MetHb formation [Bradberry 2003]. Perinatal administration of higher doses of methylene blue (4 mg/kg), given amniotically, has been reported to induce hemolysis and methemoglobinemia in infants without G6PD deficiency [Wright et al. 1999; Nelson and Hostetler 2003].

Treatment Alternatives	Clinicians have tried various treatment alternatives for severe, life-threatening methemoglobinemia, especially when the patient responds poorly to methylene blue therapy (i.e. G6PD deficiency, Hemoglobin M) [McKenzie 2010; Skold et al. 2011; McDonagh et al. 2013]. • These treatment options include exchange transfusion and hyperbaric oxygen therapy [Harrison 1977; Mier 1988; Nelson and Hostetler 2003; DeBaun et al. 2011; Skold et al. 2011]. • During treatment in the hyperbaric chamber, sufficient oxygen can be dissolved directly in the blood to support life; reversible binding to hemoglobin is not required [Wright et al. 1998; Hunter et al. 2011; McDonagh et al. 2013]. • Ascorbic acid and vitamin E (alpha-tocopherol) have been investigated, given their role in cellular detoxification, but do not seem promising as treatments for acute poisoning [Bradberry 2003; Skold et al. 2011; McDonagh et al. 2013]. • In vitro efficacy of N-acetylcysteine in reducing methemoglobinemia has been demonstrated [Wright et al. 1998]; however, its use in acute methemoglobinemia requires more study [Skold et al. 2011]. • Cimetidine (P450 inhibitor) has reduced the incidence of methemoglobinemia in patients taking dapsone, but its role in the acute care setting is unclear [Skold et al. 2011]. Exchange transfusion is typically reserved for patients in whom methylene blue treatment is ineffective (i.e., Hemoglobin M) or where contraindicated (i.e. patients on serotonin uptake inhibitors, etc.) [Ramsey et al. 2007; Khavandi et al. 2008].
Key Points	• Many patients with asymptomatic methemoglobinemia require only supportive care. • Methylene blue is an effective antidote for most patients with methemoglobinemia. • For severe methemoglobinemia, or when the patient responds poorly to methylene blue therapy, alternate treatment options include exchange transfusion and hyperbaric oxygen therapy.

Progress Check

21. The best course of action after giving the first dose of methylene blue is to

    A. Discharge the patient.
    B. Discontinue oxygen therapy.
    C. Double the second dose.
    D. Reassess the patient's clinical status and MetHb levels.

    To review relevant content, see "Methylene Blue" in this section.

22. Which of the following is a known relative contraindication for methylene blue therapy in non-life threatening cases?

    A. Glucose-6-phosphate Dehydrogenase Deficiency (G6PD).
    B. Glycogen Branching Enzyme Deficiency (GBED).
    C. Leukopenia.
    D. Fever.

    To review relevant content, see "G6PD Deficiency" in this section.

23. Which of the following is ***not*** a treatment modality for methemoglobinemia?

    A. Exchange transfusion.
    B. Hyperbaric oxygen.
    C. Methylene blue.
    D. Dapsone.

    To review relevant content, see "Methylene Blue" and "Treatment Alternatives" in this section.

What Instructions Should Be Given to Patients to Prevent Overexposure to Nitrates and Nitrites?

Learning Objective

Upon completion of this section, you will be able to

- Describe care advice the clinician can provide to

	patients to prevent overexposure to nitrates and nitrites.
Introduction	By utilizing effective risk communication techniques, the clinician can promote patient behaviors that may reduce risk of nitrate/nitrite overexposure and exposure related adverse health effects. The clinician can provide advice on • Self-care, so that patients can minimize risk of nitrate/nitrite overexposure and • When to follow-up with a health care provider. There are potential health benefits and risks from dietary sources of nitrates and nitrites. Most health risks from overexposure to nitrates and nitrites occur in susceptible populations. Preventive messages targeted to at risk populations are the key in preventing adverse health effects from overexposures.
Self-care Advice	Self-care advice creates awareness and suggests actionable behaviors that may reduce the risk of nitrate/nitrite overexposure and exposure related adverse health effects.

Sample Message	Rationale
• Have private wells used as the household water source tested for nitrates/nitrites. • Do not use untested private well water to dilute infant formula. • Contact local or state health department for private well water testing recommendations.	The American Academy of Pediatrics (AAP) consensus panel recommends that all prenatal and well-infant visits need to include questions about the home water supply. If a private well is the water source, the water should be tested for nitrates [Greer and Shannon 2005]. State and local health or environmental departments often test for nitrates, total coliforms, fecal coliform, volatile organic compounds, pH as well as any other substances that may be of concern locally. In addition, health departments or county governments should have a list of the state-certified (licensed) laboratories that test for a variety of substances.
• In addition to local or state health departments, there are many informational sources available on drinking water and private well water testing.	The local or state health department can provide recommendations for private well water testing. Other online resources regarding private wells and private well water are available. CDC's online resources on well water include:

	• Use of a non-contaminated water source is recommended until test results are available.	http://www.cdc.gov/healthywater/drinking/private/wells/testing.html http://www.cdc.gov/healthywater/drinking/private/index.html Another informational resource is the EPA Safe Drinking Water Hotline at (800) 426-4791. Bottled water is less likely to have high levels of nitrates. The standards for bottled water are set by the United States Food and Drug Administration (FDA). The FDA bases its standards on the EPA standards for tap water. If you have questions about bottled water, make sure you are informed about where your bottled water comes from and how it has been treated. http://www.cdc.gov/healthywater/drinking/bottled/index.html
	• Don't feed infants less than 3 months of age home-prepared infant food from certain vegetables. • It is okay to feed infants commercially prepared infant foods.	The American Academy of Pediatrics (AAP) consensus panel concluded that • Home-prepared infant foods from vegetables (e.g., spinach, beets, green beans, squash and carrots) should be avoided until infants are 3 months of age or older. • Infants fed commercially prepared infant food are general not at risk of

	nitrate poisoning [Greer and Shannon 2005].
• Breastfeeding should continue.	Breastfed infants are not at risk of excessive nitrate exposure from mothers who ingest water with a high nitrate content (up to 100 ppm nitrate nitrogen) because the nitrate concentration does not increase significantly in breast milk [Greer and Shannon 2005].
• Reduce the amount of cured and processed meats in diet.	Nitrates and nitrites are used in meat products including • Bacon, • Bologna, • Corned beef, • Hot dogs, • Luncheon meats, • Sausages and canned and cured meat, and • Hams. Levels of nitrates and nitrites used in meat production and packaging are regulated by the FDA and USDA. An Expert Panel representing the American Institute for Cancer Research recommends reducing the consumption of cured and processed meats to avoid adverse health effects. However a safe consumption level is not specified [WCRF 2007].

	• Eat a variety of colors and types of vegetables (4-5 servings/day) and fruits (4-5 servings/day).	Vegetable and fruit consumption has health benefits and studies have indicated that plant-based nitrates and nitrites play essential physiologic roles in supporting cardiovascular health and gastrointestinal immune function [Hord 2011]. The American Heart Association and other private health agencies recommend adherence to the public dietary health recommendations in the United States [Appel et al. 2006]. A dietary chart can be accessed at: http://www.heart.org/HEARTORG/GettingHealthy/NutritionCenter/HealthyDietGoals/Suggested-Servings-from-Each-Food-Group_UCM_318186_Article.jsp
Advice On When to Follow-up With a Health Care Provider	Patients should be advised to consult their physician if they or their child develop signs or symptoms to include • Changes in skin or mucous membrane color (particularly blue color or cyanosis), • Difficulty breathing, • Gastrointestinal disturbances such as nausea, severe diarrhea, vomiting, • Dehydration, • Rapid pulse, or • Decreased level of consciousness.	
ATSDR Patient Education Care Instruction Sheet	ATSDR has developed a patient education care instruction sheet on nitrates/nitrites toxicity that you might find useful. It can be found at http://www.atsdr.cdc.gov/csem/nitrate_2013/docs/nitrate_patient-education.pdf.	

Key Points	• Patients should be instructed on ways to protect themselves from over exposure to nitrates and nitrites that might increase their risk of exposure related adverse health effects. • All prenatal and well-infant visits should include questions about the home water supply. If the water source is a private well, the water should be tested for nitrates in addition to other substances depending on area conditions. For more information on well water testing and maintenance, individuals should contact their local or state health department. • Home-prepared infant foods from vegetables (e.g., spinach, beets, green beans, squash and carrots) should be avoided until infants are 3 months of age or older. • Limiting the consumption of processed or cured meats may decrease the risk of adverse health effects from overexposure to nitrates/nitrates. • US public dietary health recommendations for consumption of fruits and vegetables should be promoted for their health benefits.
Progress Check	24. Which of the following instructions regarding exposure to nitrates and nitrites is/are true? A. Limit the consumption of processed or cured meats. B. Infants under 3 months of age should not be fed home prepared foods containing vegetables. C. Infants fed commercially prepared infant food are general not at risk of nitrate poisoning D. Households using private wells for drinking water should have the well tested for nitrates. E. All of the above. F. A, B, and D only. *To review relevant content, see "Introduction" and "Self Care Advice" in this section.*

Sources of Additional Information

Specific Information	Please refer to the following Web resources for more information on the adverse effects of Nitrates/Nitrites,

the treatment of Nitrates/Nitrites associated diseases, and management of persons exposed to Nitrates/Nitrites.

- Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov
  - For chemical, emergency situations
    - CDC Emergency Response: 770-488-7100 and request the ATSDR Duty Officer
  - For chemical, non-emergency situations
    - CDC-INFO http://www.cdc.gov/cdc-info/
    - 800-CDC-INFO (800-232-4636) TTY 888-232-6348 - 24 Hours/Day
    - E-mail: cdcinfo@cdc.gov

      PLEASE NOTE:
      ATSDR cannot respond to questions about individual medical cases, provide second opinions or make specific recommendations regarding therapy. Those issues should be addressed directly with your health care provider.
  - ATSDR ToxFAQs for Nitrates/Nitrites http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=1186&tid=258

- Centers for Disease Control and Prevention
  - Well Testing Information http://www.cdc.gov/healthywater/drinking/private/wells/testing.html
  - Healthy drinking water http://www.cdc.gov/healthywater/drinking/private/index.html

- U.S. Environmental Protection Agency (EPA)
  - http://www.epa.gov
  - EPA Safe Drinking Water Hotline at (800) 426-4791.

	o Well Water Information Based on Where You Live http://water.epa.gov/drink/info/well/whereyoulive.cfm o State Certified Drinking Water Laboratories o http://water.epa.gov/scitech/drinkingwater/labcert/statecertification.cfm o Bottled Water Basics http://water.epa.gov/drink/info/upload/2005_09_14_faq_fs_healthseries_bottledwater.pdf o Source Water Protection http://water.epa.gov/infrastructure/drinkingwater/sourcewater/protection/index.cfm o EPA Basic Information About Nitrates in Drinking Water http://water.epa.gov/drink/contaminants/basicinformation/nitrate.cfm o Water: Private Wells – Related Links http://water.epa.gov/drink/info/well/links.cfm
General Environmental Health Information	Please refer to the following Web resources for general information on environmental health. • Agency for Toxic Substances and Disease Registry http://www.atsdr.cdc.gov o Taking an Exposure History CSEM https://www.atsdr.cdc.gov/csem/csem.asp?csem=33&po=0 o To view the complete library of CSEMs http://www.atsdr.cdc.gov/csem/csem.html o Exposure History Worksheet http://www.atsdr.cdc.gov/csem/exphistory/docs/CSEMExposHist-26-29.pdf o ATSDR Division of Regional Operations. ▪ Through the working relationships they have established with EPA, other federal and state agencies, individual citizens, and community groups, regional representatives are able to maintain current and historic knowledge of the sites and issues in their regions. ▪ ATSDR's Regional Offices, along with the states and territories that they cover as well

as contact information, can be found at www.atsdr.cdc.gov/DRO/dro_contact.html

- ATSDR State Cooperative Agreement Program http://www.atsdr.cdc.gov/states/index.html

  - The Cooperative Agreement Program provides essential support in communities nationwide to fulfill the mission of the Agency for Toxic Substances and Disease Registry (ATSDR).
  - The program funds 30 states and one tribal government to develop and strengthen their abilities to evaluate and respond to environmental public health issues.

- Centers for Disease Control and Prevention (CDC) http://www.cdc.gov

  - CDC works to protect public health and the safety of people, by providing information to enhance health decisions, and promotes health through partnerships with state health departments and other organizations.
  - The CDC focuses national attention on developing and applying disease prevention and control (especially infectious diseases), environmental health, occupational safety and health, health promotion, prevention and education activities designed to improve the health of the people of the United States.

- National Center for Environmental Health (NCEH) http://www.cdc.gov/nceh

  - NCEH works to prevent illness, disability, and death from interactions between people and the environment. It is especially committed to safeguarding the health of populations that are particularly vulnerable to certain environmental hazards - children, the elderly, and people with disabilities.
  - NCEH seeks to achieve its mission through science, service, and leadership.

- National Institute of Health (NIH) http://www.nih.gov

  - A part of the U.S. Department of Health and Human Services, NIH is the primary Federal agency for conducting and supporting medical research.

- National Institute of Occupational Safety and Health (NIOSH) http://www.cdc.gov/niosh/

  - NIOSH is in the U.S. Department of Health and Human Services and is an agency established to help assure safe and healthful working conditions for working men and women by providing research, information, education, and training in the field of occupational safety and health.

- American College of Occupational and Environmental Medicine (ACOEM) http://www.acoem.org/

  - ACOEM is the nation's largest medical society dedicated to promoting the health of workers through preventive medicine, clinical care, research, and education.
  - Its members are a dynamic group of physicians encompassing specialists in a variety of medical practices united via the College to develop positions and policies on vital issues relevant to the practice of preventive medicine both within and outside of the workplace.

- American College of Medical Toxicologists (ACMT) http://www.acmt.net

  - ACMT is a professional, nonprofit association of physicians with recognized expertise in medical toxicology.
  - The College is dedicated to advancing the science and practice of medical toxicology through a variety of activities.

- American College of Preventive Medicine (ACPM) http://www.acpm.org

  - ACPM is the national professional society for physicians committed to disease prevention and health promotion.
  - ACPM's 2,000 members are engaged in preventive medicine practice, teaching and research.

- Association of Occupational and Environmental Clinics (AOEC) http://aoec.org

  - AOEC is a network of more than 60 clinics and more than 250 individuals committed to improving the practice of occupational and environmental medicine through information sharing and collaborative research.

- Pediatric Environmental Health Specialty Units (PEHSUs) http:///www.pehsu.net

  - The PEHSUs provide education and consultation for health professionals, public health professionals and others about the topic of children's environmental health.
  - The PEHSU staff is available for consultation about potential pediatric environmental health concerns affecting both the child and the family. Health care professionals may contact their regional PEHSU site for clinical advice.

- Poison Control Centers

  - The American Association of Poison Control Centers can be contacted for questions about poisons and poisonings. The web site provides information about poison centers and poison prevention. AAPC does not provide information about treatment or diagnosis of poisoning or research information for student papers.
  - American Association of Poison Control Centers may be contacted at 1-800-222-1222 or http://www.aapcc.org

Posttest

Instructions

To complete the assessment and posttest, go to http://www2a.cdc.gov/TCEOnline/registration/detailpage.asp?res_id=4035 and follow the instructions on that page. You can immediately print your continuing education certificate from your personal transcript online. No fees are charged.

Posttest

1. Nitrites and nitrates are

   A. Naturally occurring organic ions.
   B. Relatively insoluble in water.
   C. Ions that readily migrate in ground water.
   D. All of the above.

2. Which of the following water sources is generally most likely to contain high levels of nitrates or nitrites?

   A. Bottled water.
   B. Large municipal water supplies.
   C. Shallow, rural domestic wells.
   D. Water from deep wells.

3. Which of the following is true regarding route(s) of exposure to nitrates and nitrites in humans?

   A. The primary route of occupational exposure is ingestion.
   B. The primary route of exposure for the general population is dermal.
   C. Primary routes of exposure are the same for occupational and non-occupational populations.
   D. None of the above.

4. Which of the following subpopulations are most at risk of adverse effects from nitrate exposure?

   A. Girls age 13-18 years old.
   B. Telephone line workers.
   C. The elderly.

D. Infants younger than 4 months of age.
E. Individuals with anemia.

5. Which of the following are possible sources of nitrate exposure?

   A. Certain topical burn medications.
   B. Shallow domestic wells in rural areas.
   C. Meat preservatives.
   D. Seepage from septic tanks.
   E. All of the above.

6. Which statement about nitrates is true?

   A. Nitrates can be converted into more toxic nitrites in the gut.
   B. The higher alkalinity of an infant's gut protects it from nitrate toxicity.
   C. Vomiting and diarrhea do not affect the absorption of nitrates or nitrites.
   D. No case of nitrate poisoning has been reported since 1950.
   E. Adults are immune from nitrate toxicity if they drink water from public water systems.

7. Which of the following is/are true?

   A. The present maximum contaminant level for nitrates in drinking water appears to adequately protect even sensitive populations from nitrate-induced toxicity.
   B. Some domestic and public-supply wells in the United States have nitrate levels above the U.S. EPA drinking water standard of 10 parts per million.
   C. The acceptable daily intake (ADI) for nitrate is 3.7 mg/kg/day or 222 mg nitrate per day for a 60 kg adult.
   D. All of the above.
   E. None of the above.

8. All of the following are true regarding dietary nitrate intake **EXCEPT**

A. Vegetables account for about 80% of nitrates in a typical human diet.
B. Nitrate levels vary by type of vegetable with higher levels generally found in leafy vegetables.
C. There are benefits from dietary intake of nitrates and nitrites.
D. Cured and processed meats account for about 85% nitrates in a typical human diet.
E. Nitrates in drinking water should not exceed 10 ppm.

9. The toxicity of nitrates is enhanced by in vivo conversion to

A. Urea.
B. CO_2.
C. Protein.
D. Nitrites.

10. Effects of methemoglobinemia include which of the following?

A. Cyanosis.
B. Coma or convulsions.
C. Dysrhythmias.
D. All of the above.

11. Methemoglobinemia can be induced by which of the following?

A. Chloroquine.
B. Lidocaine.
C. Nitroglycerine.
D. Dapsone.
E. All of the above.

12. Which of the following systems is most directly affected by nitrates?

A. Reproductive system.
B. Hematologic system.
C. Neurological system.
D. Immune system.

13. Which statement is true?

    A. Signs and symptoms of methemoglobinemia are precisely correlated with percent total oxidized hemoglobin.
    B. Fetal hemoglobin is less readily oxidized by nitrites to methemoglobin than is adult hemoglobin.
    C. Methemoglobin causes arterial blood to be bright red in color.
    D. Standard pulse oximetry is the most useful diagnostic test for nitrate toxicity.
    E. None of the above.

14. What key areas should be addressed in the exposure history?

    A. Recent use of medications by infant and mother.
    B. Type of formula, feeding regimen, and source of dilution water.
    C. Drinking water source and supply.
    D. All of the above.

15. Which of the following is/are true regarding the clinical assessment?

    A. All cyanotic patients should be assessed for possible cardiac and lung disease (cardiac murmurs, gallops, arrhythmias, rales, rhonchi, wheezes, dullness, or hyperresonance in the chest).
    B. A central chocolate brown or slate gray cyanosis that does not respond to administration of 100% oxygen is suggestive of methemoglobinemia.
    C. The victim is often less ill than one would expect from the severity of 'cyanosis' (but not always)
    D. All of the above.

16. Useful diagnostic test(s) for nitrate toxicity include which of the following?

    A. Measurements of nitrates in blood, urine, or saliva.

B. Measurements of nitrites in blood, urine, or saliva.
C. Blood methemoglobin level.
D. All of the above.

17. Which of the following treatments can be used for patients with nitrate toxicity?

A. Hyperbaric oxygen therapy.
B. Methylene blue.
C. 100% oxygen.
D. Exchange transfusion.
E. All of the above.

18. What condition is a relative contraindication to methylene blue treatment, especially in cases of non-life threatening methemoglobinemia?

A. Psoriasis.
B. G6PD deficiency.
C. Methemoglobinemia.
D. Diarrhea and vomiting.
E. None of the above.

19. Which of the following instructions regarding exposure to nitrates and nitrites is **FALSE**?

A. Limit the consumption of processed or cured meats.
B. Infants under 3 months of age should not be fed home prepared foods containing vegetables.
C. Infants fed commercially prepared infant foods containing vegetables are at increased risk of nitrate poisoning.
D. Households using private wells for drinking water should have the well tested for nitrates.

Relevant Content	To review content relevant to the posttest questions, see
Question	**Location of Relevant Content**
1.	What are nitrates/nitrites?

	• Describe what nitrates and nitrites are.
2.	Where are nitrates and nitrites found? • Identify sources of nitrates and nitrites.
3.	What are routes of exposure for nitrates and nitrites? • Describe the primary routes of exposure to nitrates and nitrites.
4.	Who is at risk of adverse health effects from overexposure to nitrates and nitrites? • Identify the population most susceptible to adverse health effects from overexposure to nitrates and nitrites.
5.	Where are nitrates and nitrites found? • Identify sources of nitrates and nitrites.
6.	What is the biologic fate of nitrates and nitrites in the body? • Describe what happens to nitrates and nitrites once they enter the body. What are the health effects from exposure to nitrates and nitrites? • Describe mechanisms contributing to health effects from exposure to nitrates and nitrites.
7.	What are U.S. standards and regulations for nitrate and nitrite exposure? • Describe the U.S. Environmental Protection Agency's (EPA's) recommended limit for nitrates and nitrites in drinking water.
8.	Where are nitrates and nitrites found? • Identify sources of nitrates and nitrites.

	What are the U.S. standards and regulations for nitrate and nitrite exposure? • Describe the EPA's recommended limit for nitrates and nitrites in drinking water. • Describe the FDA's recommended limit for nitrates and nitrites in bottled water and foodstuffs.
9.	What is the biologic fate of nitrates and nitrites in the body? • Describe what happens to nitrates and nitrites once they enter the body. What are the health effects from exposure to nitrates and nitrites? • Describe mechanisms contributing to health effects from exposure to nitrates and nitrites.
10.	What are the health effects from exposure to nitrates and nitrites? • Describe the health effects from exposure to nitrates and nitrites.
11.	How should patient's potentially overexposed to nitrates and nitrites be evaluated? • Describe the clinical assessment of an infant overexposed to nitrates and nitrites.
12.	What are the health effects from exposure to nitrates and nitrites? • Describe the health effects from exposure to nitrates and nitrites. How should patient's potentially overexposed to nitrates and nitrites be evaluated? • Describe the signs and symptoms of methemoglobinemia.

13.	What are the health effects from exposure to nitrates and nitrites? • Describe the mechanisms contributing to health effects from exposure to nitrates and nitrites. • Describe the health effects from exposure to nitrates and nitrites. How should patient's potentially overexposed to nitrates and nitrites be evaluated? • Describe the clinical assessment of an infant overexposed to nitrates and nitrites. • Describe the signs and symptoms of methemoglobinemia. What laboratory tests can assist with diagnosis of nitrate and nitrite toxicity? • Identify the laboratory test results that indicate methemoglobinemia.
14.	How should patient's potentially overexposed to nitrates and nitrites be evaluated? • Describe the clinical assessment of an infant overexposed to nitrates and nitrites.
15.	How should patient's potentially overexposed to nitrates and nitrites be evaluated? • Describe the clinical assessment of an infant overexposed to nitrates and nitrites. • Describe signs and symptoms of methemoglobinemia.
16.	What laboratory tests can assist with diagnosis of nitrate/nitrite toxicity? • Identify laboratory test results that indicate methemoglobinemia.
17.	How should patients exposed to nitrates and nitrites be treated and managed?

	• Describe treatment modalities in the management of methemoglobinemia caused by nitrate and nitrite toxicity.
18.	How should patients exposed to nitrates and nitrites be treated and managed? • Describe treatment modalities in the management of methemoglobinemia caused by nitrate and nitrite toxicity.
19.	What instructions should be given to patients' potentially overexposed to nitrates and nitrites? • Describe care advice the clinician can provide to patients to prevent overexposure to nitrates and nitrites.

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Table of Tables and Figures

Table of Figures

Table of Tables

4 Suggested Lab Tests for Methemoglobinemia Workup