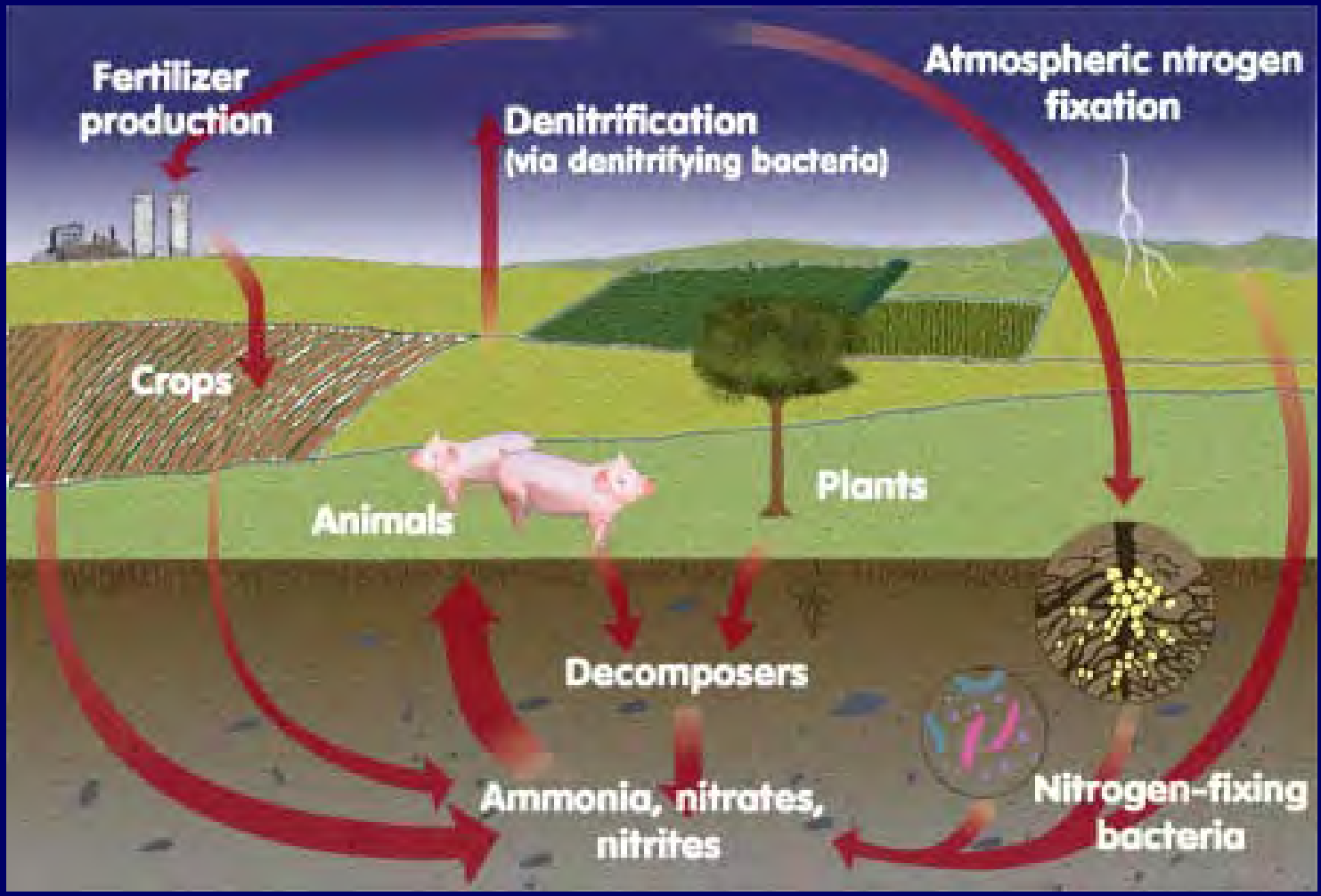
Nitrate Poisoning in Ruminants

Nitrogen Cycle



Introduction

Humans add N to replenish soils

Nature varies the amount of N depending on environmental factors

When livestock consume excess N (water or feed), usually termed nitrate poisoning.

Historical Perspective

Historically, acute nitrate/nitrite poisoning first reported over 100 yrs ago in connection with deliberate attempts to poison sheep on disputed ranges.

"Salting the Range" to get rid of "range maggots"

- Ranchers spread KNO_3 to poison sheep on "cattle" ranges

Historical Perspective

Cornstalk Poisoning 1895 Kansas

DECEMBER, 1942 CIRCULAR 214

AGRICULTURAL EXPERIMENT STATION

KANSAS STATE COLLEGE OF AGRICULTURE
AND APPLIED SCIENCE
MANHATTAN, KANSAS

DEPARTMENT OF VETERINARY MEDICINE

DISEASES OF FEEDER CATTLE IN KANSAS[1]

BY HERMAN FARLEY

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Historical perspectives (cont.)

Oat hay poisoning was known for years before being recognized by Colorado and Wyoming workers in 1939 as a nitrate/nitrite acute intoxication.

GENERAL DISEASES

"CORNSTALK DISEASE"

"Cornstalk disease" is a mysterious ailment which causes sudden death in cattle while feeding in cornstalk fields late in the fall or early winter.

From a disease producing standpoint the term "cornstalk disease" is meaningless, but it serves to explain in a general way certain fatalities which cannot be explained otherwise.

This disease is restricted to those sections where farmers harvest their corn by picking the ears from the standing stalks, and then turn their cattle into the cornstalk fields. Apparently it is limited in its distribution to the middle and northern portions of the Mississippi Valley.

The disease frequently causes death in animals before its presence is suspected. The cattle appear to be in perfect condition before they are turned into the stalk field, but the following morning one or more of the herd may be found dead. Sometimes after losing a few cattle no further losses may occur in the herd. Postmortem examination does not furnish any conclusive or satisfactory evidence as to the cause of death.

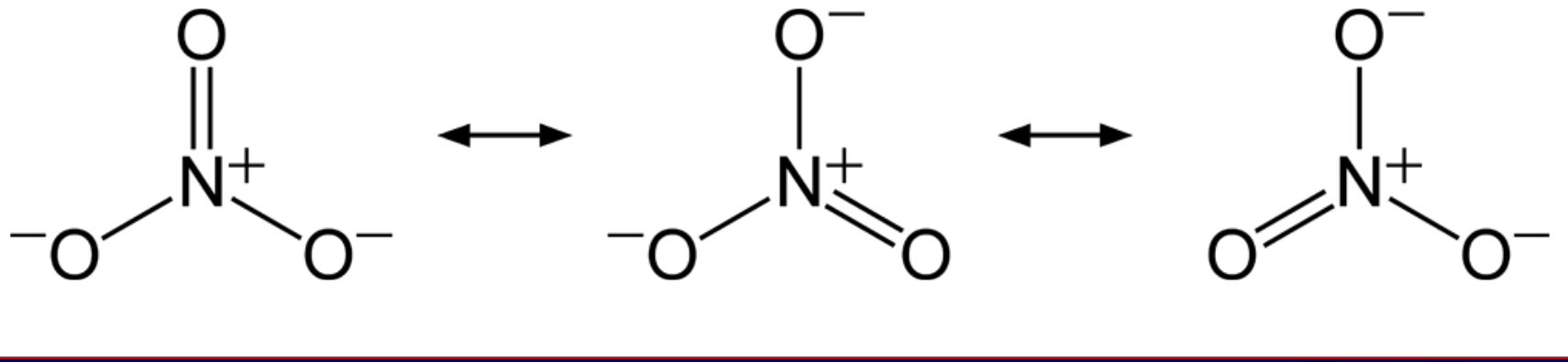
Cause: The cause of the disease is undetermined, but due to its sudden onset and rapid termination investigators are inclined to believe that the causative factor may be a rapid-acting poison developed in the stalks in the same manner as prussic acid is developed in other plants known to be capable of producing this poison.

Symptoms: The disease comes on rather suddenly with few advanced symptoms. If the cattle are in the stalk field, the affected animal is noticed lying or standing apart from the herd. Sometimes it may become nervous, and apparently develops central nervous disturbances as the disease progresses. The symptoms of suffering and delirium are followed by a complete loss of consciousness and death. Death usually takes place within 24 hours after the first symptoms are noticed.

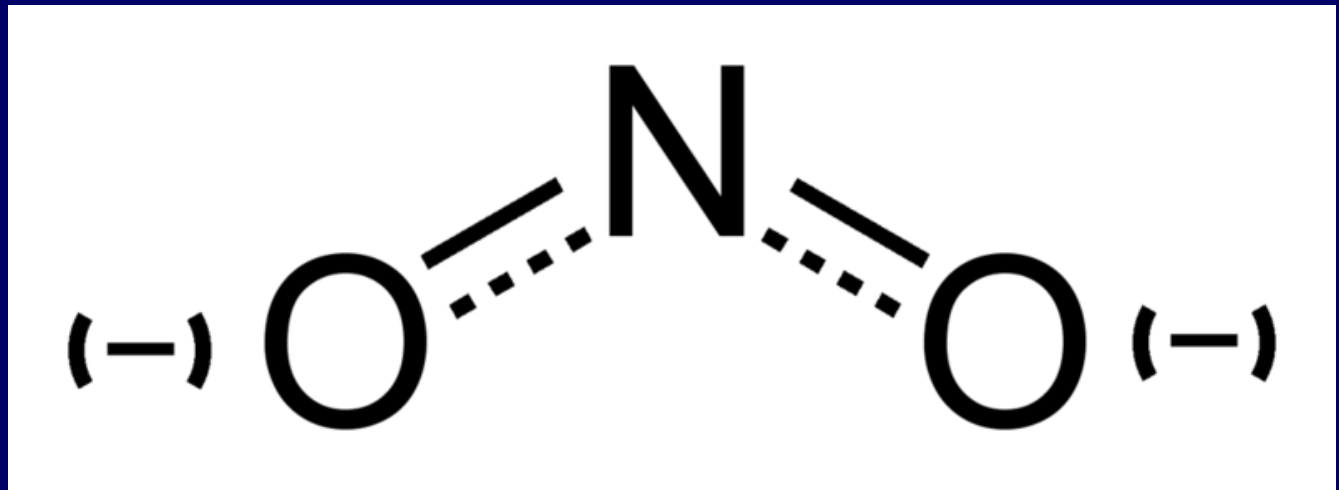
Root of the problem

Acute nitrate/nitrite poisoning is caused by the presence of nitrite in the blood at a level sufficient to cause anoxia (internal suffocation). Nitrate (NO_3^-) can be reduced to nitrite (NO_2^-) by the microorganisms in the gastrointestinal tract at a rate that overwhelms the body's defense system

Nitrate (NO_3)



Nitrite (NO_2)



Converting or Comparing Various Nitrate Reports

In analysis for nitrate, the actual element determined is the oxidized nitrogen. However, values may be reported as percent potassium nitrate (KNO_3 [in gunpowder; saltpeter; N from urine]), nitrate (NO_3), or nitrate-nitrogen (NO_3-N).

Reports may be given as potassium nitrate, nitrate or nitrate-nitrogen and, likewise, may be reported as either percent or as parts per million (ppm).

Nitrate buildup in plants

Under normal growth conditions there is little nitrate buildup in the plant even though the plant's roots are absorbing large amounts of nitrate, because the stem and leaves normally convert (i.e., reduce) nitrate to protein (ammonia then amino N) about as fast as it is absorbed by the roots.

Under certain conditions, however, this balance can be disrupted so that the roots will accumulate nitrate faster than the plant can convert the nitrate to protein.

Nitrate Buildup in Plants

The nitrate-to-protein cycle in a plant is dependent on three factors:

- Adequate water
- Energy from sunlight
- A temperature conducive to rapid chemical (enzymatic) reactions

Environmental Factors & Drought

N-reductase is a key enzyme.

When established plants begin growth after moderate drought, high levels of N may be available for uptake

Moderate drought = slow growth & little leaching (i.e., more nitrate in soil)

Environmental Factors & Drought

- N uptake at night or during cloudy weather decreases N reductase conc.
  - Shading reduces N reductase activity
  - Shading reduces Photosynthesis so Carbohydrate intermediates are lacking
- Plants take up Nitrate during night when soil is typically more moist.
- This Nitrate may not be reduced to Nitrite and Amino N during daylight because of reduced enzyme activity

Plant uptake of Nitrates

Factors that facilitate uptake of nitrate by plants include:

- use of nitrogen-containing fertilizers;
- areas where stock have congregated and urinated/defecated (e.g. barnyards);
- Use of heavy manure applications

Nitrate uptake in Plants

Other environmental factors that may increase nitrate supply in plants (alter N reductase concentrations):

- herbicide application — especially phenoxy herbicides such as 2,4-D

- wilting

Plants likely to have excessive N buildup

Crops capable of high levels of nitrate accumulation under adverse conditions include corn, small grains, sudangrass, and sorghum.

Weeds capable of nitrate accumulation include pigweed, lambsquarter, sunflower, bindweed and many others.

Vegetables capable of accumulating large amounts of nitrate that are most frequently grazed include sugar beets, lettuce, cabbage, potatoes and carrots.

Plant Families

Lambsquarters	Chenopodiaceae
Pigweed	Amaranthaceae
Sunflower	Asteraceae
Mustard	Cruciferae
Potato	Solanaceae
Grasses	Poaceae (monocots)

Nitrate Toxicity

Under normal conditions, nitrate ingested by ruminant livestock like cattle, sheep and goats is converted to ammonia in the rumen by bacteria.

The steps of conversion in this process are as follows:

Nitrate (NO_3) —> Nitrite (NO_2) ——> Ammonia (NH_3) ——> Amino Acid ——> Protein

Cause of death

Nitrate is converted to nitrite faster than nitrite is converted to ammonia. Consequently, when higher than normal amounts of nitrate are consumed, an accumulation of nitrite may occur in the rumen.

Nitrite then will be absorbed into the bloodstream and will cause hemoglobin to be converted to methemoglobin, which is unable to transport oxygen.

Thus, when an animal dies from nitrate poisoning, it is due to a lack of oxygen.

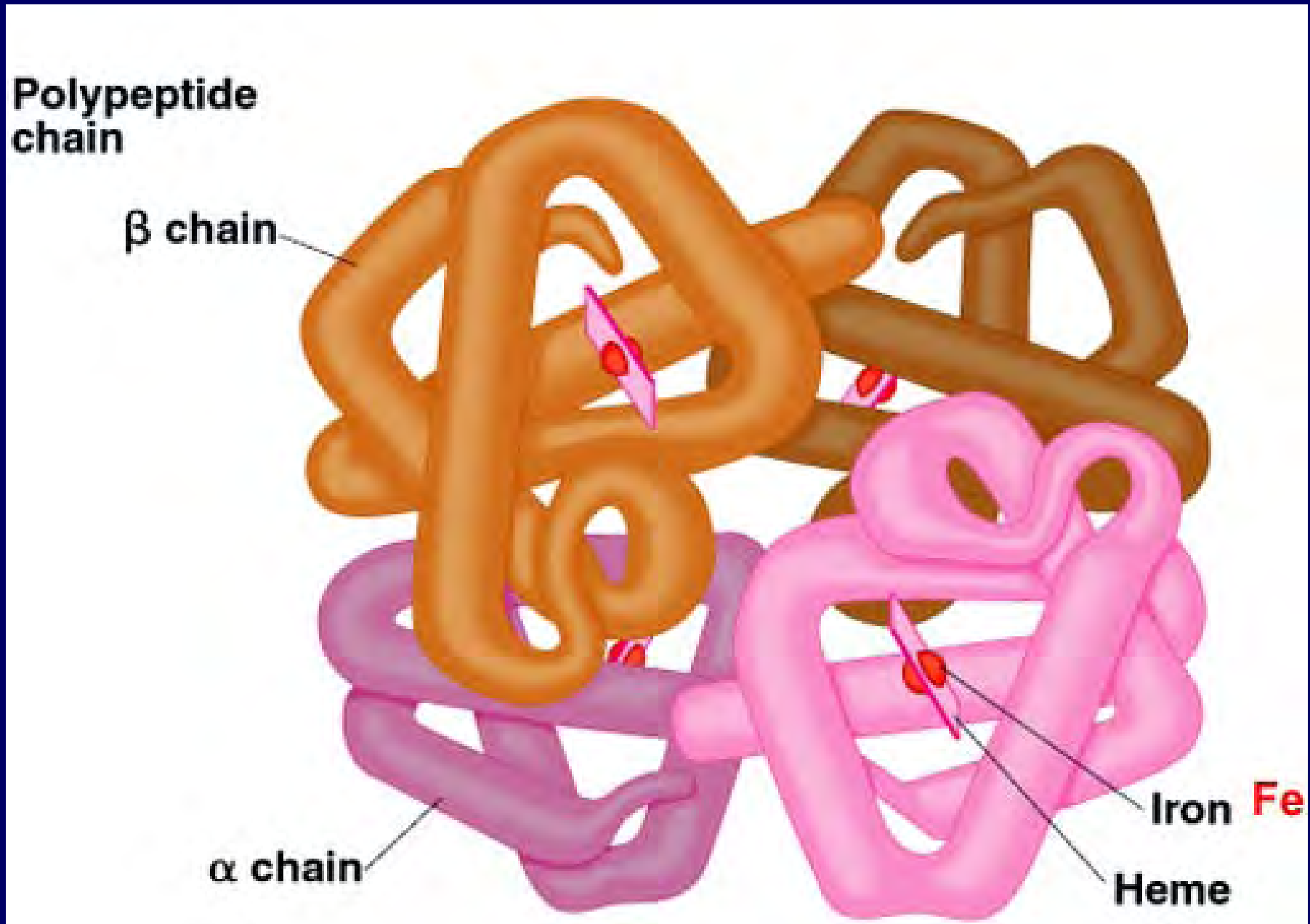
Hb to MetHb

Nitrite combines with the hemoglobin of the red blood cells to form methemoglobin. Methemoglobin cannot transport oxygen to the body tissues.

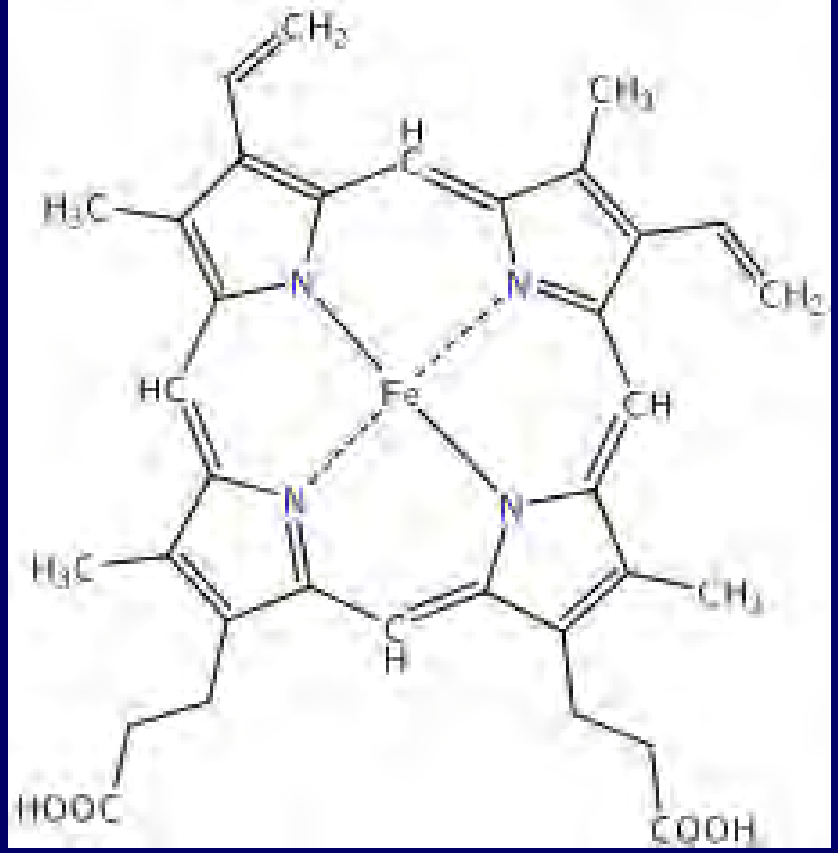
Methemoglobin is hemoglobin that has been oxidized from the ferrous (Fe++) to the ferric (Fe+++) state, thus unable to bind oxygen. NADH- methemoglobin reductase enzyme reduces methemoglobin to hemoglobin. Methemoglobinemia results from either inadequate enzyme activity or too much methemoglobin production.

Hemeglobin:

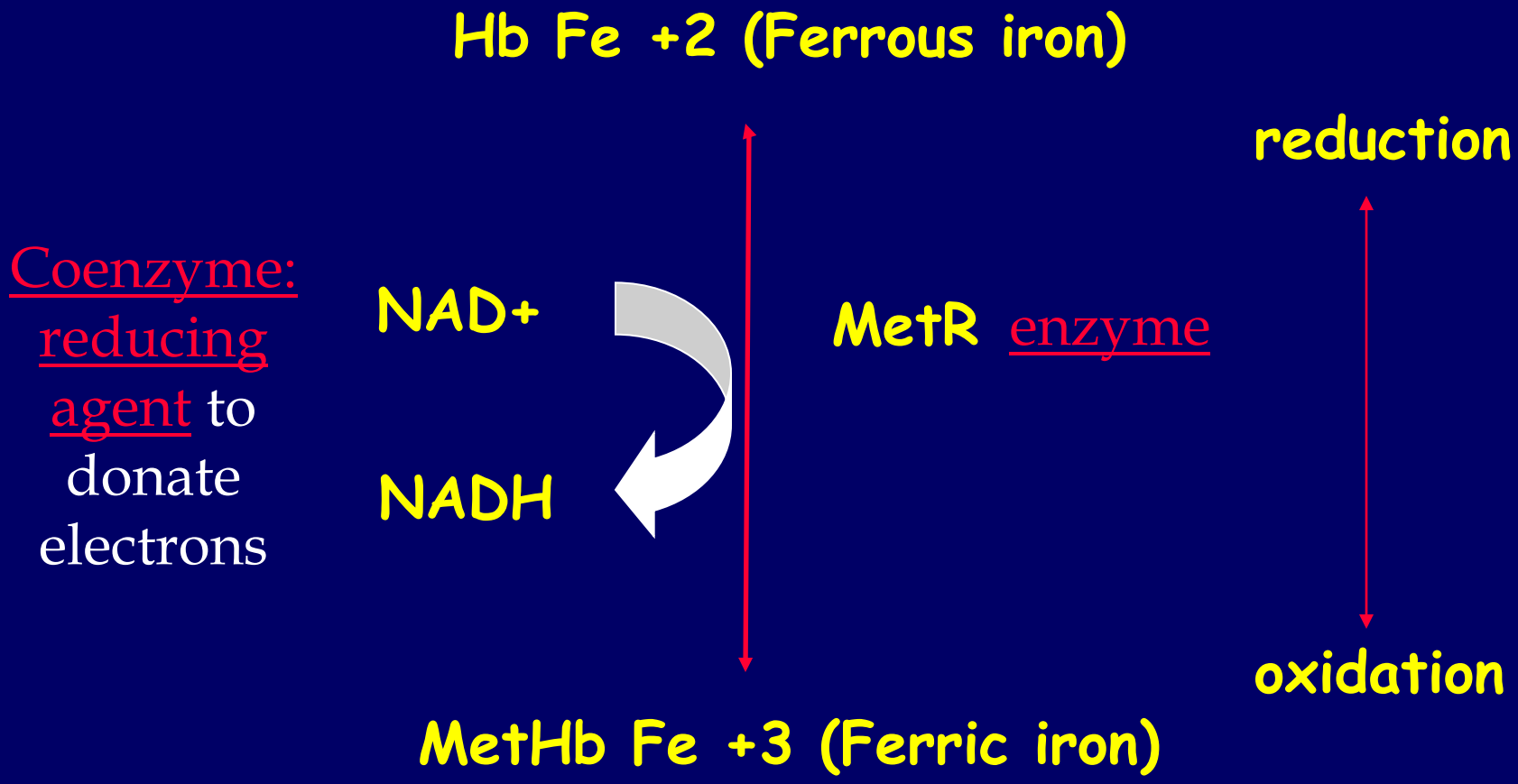
Each heme group contains one iron atom, that can bind one oxygen molecule. The most common type of hemoglobin in mammals contains four such subunits.



(b) Hemoglobin



The iron ion may either be in the Fe^{2+} or Fe^{3+} state, but methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily oxidizes Fe to (Fe^{3+}), so iron must exist in the +2 oxidation state in order to bind oxygen. The enzyme methemoglobin reductase reactivates hemoglobin found in the inactive (Fe^{3+}) state by reducing the iron center.


Hb Fe +2 (Ferrous iron)
reduction
Coenzyme: reducing agent to donate electrons
NAD+
NADH
MetR enzyme
oxidation
MetHb Fe +3 (Ferric iron)

Primary Symptoms

The chief symptom, anoxia or oxygen deprivation, causes a bluish color of the mucous membranes of stricken animals.

The blood is chocolate-brown in color due to methemoglobin formation. These characteristics are indicative of acute nitrate/nitrite poisoning.

Maximum levels of MetHb may occur 4-6 hours after eating.

General rule of thumb: Signs of toxicity @10-20% MetHb

Death at 75-80% Met Hb

MetHb vs. normal blood

Why is the blood on the left brownish while the blood on the right is red in color?

Clinical signs

Signs of nitrate poisoning are:

- diarrhea and vomiting;
- salivation;
- abdominal pain.

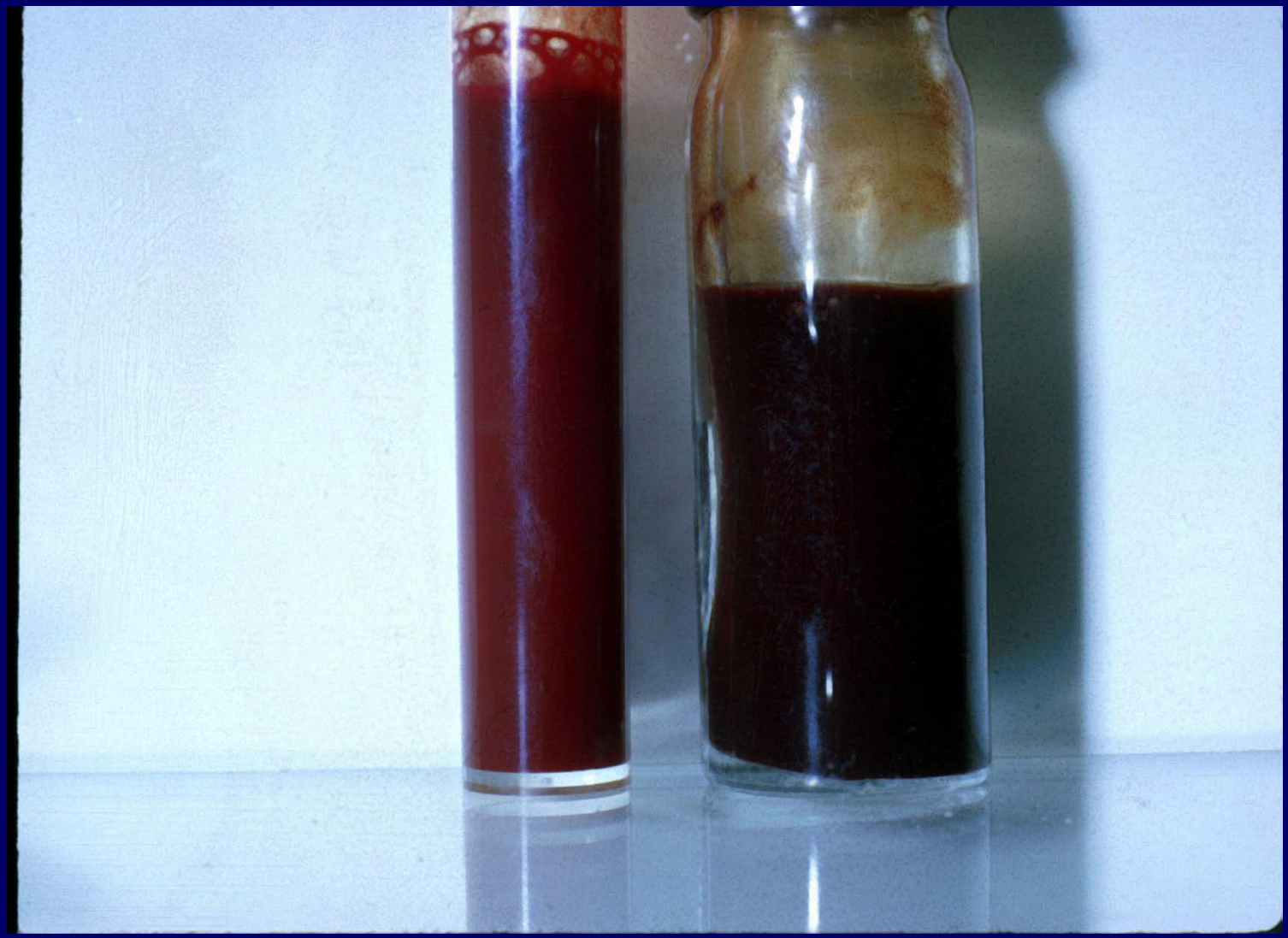
Nitrate is not very toxic

Nitrates have a direct, caustic effect on the lining of the gut if consumed in large quantities.

Clinical signs (Acute toxicity)

Signs of nitrite poisoning usually appear 3–8 hours after the toxic material is consumed. These include:

- rapid, noisy and difficult breathing;
- blue/chocolate-colored mucous membranes (muzzle; vulva in females);
- rapid pulse;
- salivation, bloat, tremors, staggering;
- dark, chocolate-colored blood.
- weakness, coma, terminal convulsions, death.



Other indications of Nitrate Toxicity (Chronic)

Abortions — pregnant females that survive nitrate/nitrite poisoning may abort due to a lack of oxygen to the fetus;

- abortions usually occur 10–14 days after exposure to nitrates;

Vit A deficiency

Reduced weight gains

Milk production reduced

Hypothyroidism

Variation in Toxicity

Factors making nitrate less toxic include:

- Animals can be conditioned to eat larger amounts of feed with a high nitrate content if the increase is gradual (2-3 days).
  - within 4 hours get increases in nitrate reducing microbe pop'n
  - withdraw nitrate and takes 3 weeks for pop'n to return to baseline levels
- Healthy animals are less likely to be adversely affected than animals in poor health.
- Adequate amounts of available carbohydrates (grain) allow the animal to consume more nitrate because carbohydrates enhance the conversion from nitrate to microbial protein- promotes rapid fermentation.

Variation in Toxicity

Factors making nitrate more toxic include:

- Rapid diet changes can trigger nitrate poisoning.
- Parasitism or other conditions causing anemia will increase susceptibility.
- Nitrate in more than one diet component (e.g. water and forage) can exceed toxic limits.

Diagnosis

Diagnosis is based on:

- observed clinical signs;
- possible exposure to toxic plants, feeds or water;
- post-mortem findings;
- laboratory tests.

Post-mortem findings

From nitrate poisoning:

- severe reddening and stripping of the stomach and intestinal linings.

From nitrite poisoning:

- dark red or chocolate-brown blood that clots poorly;
- pinpoint hemorrhages in internal organs and on internal surfaces;
- accumulation of blood in the stomach wall.

Species differences

There is considerable variation between species in their susceptibility to nitrite poisoning.

Susceptibility depends on GI tract activity

- All reduction in Nitrate to Nitrite is attributed to microorganisms in GIT

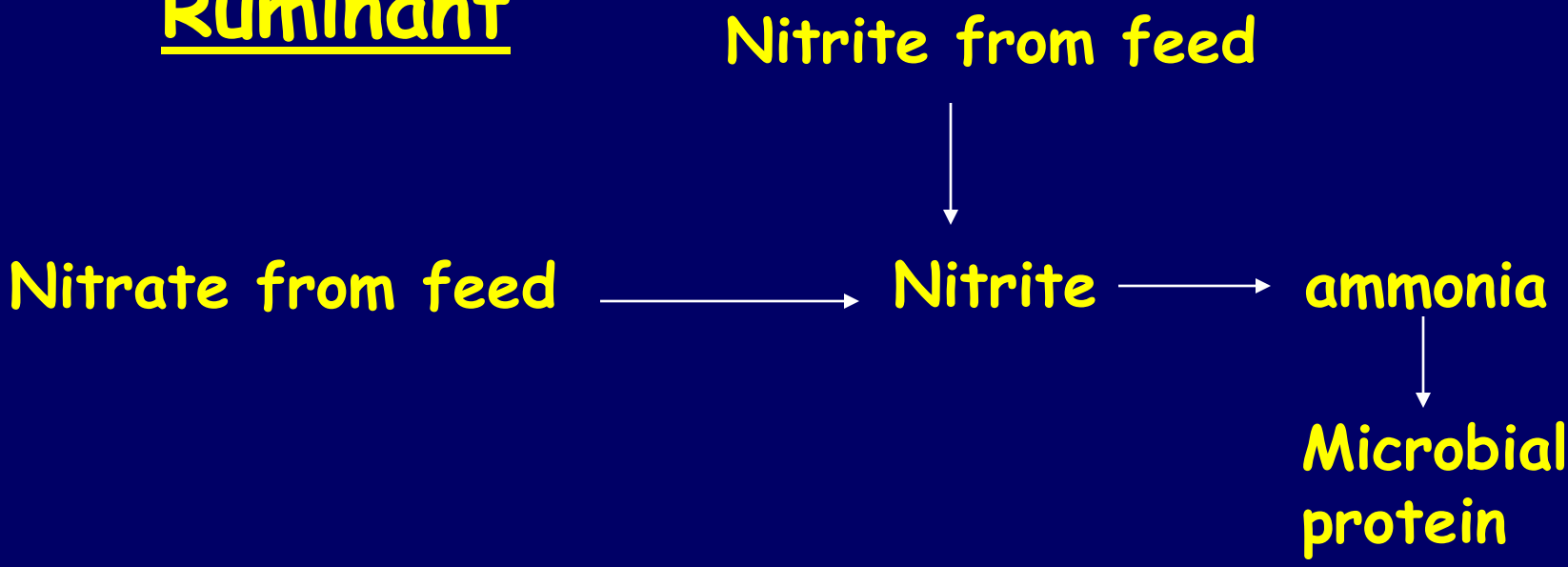
Pigs are the most susceptible, then cattle, sheep, and then horses.

Species differences

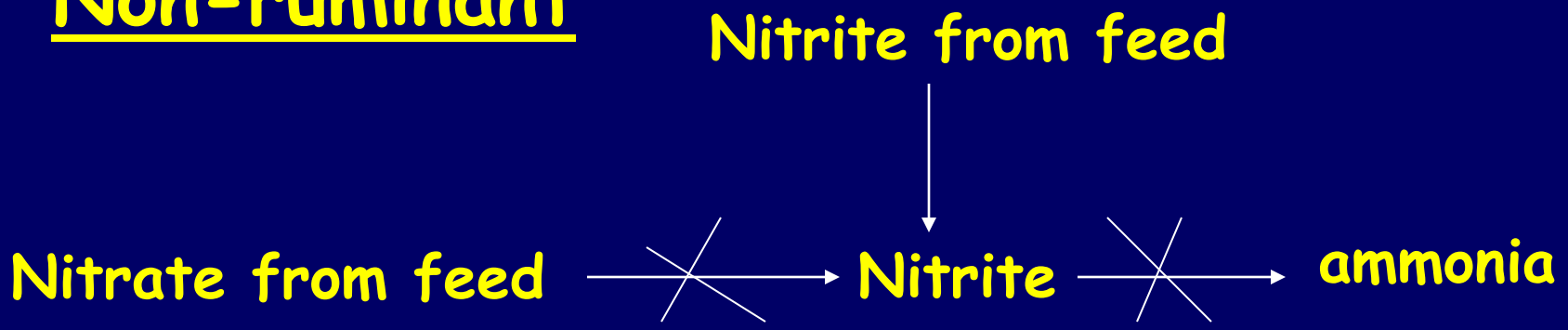
Non-ruminants have no mechanism for converting nitrate to nitrite in their digestive tracts, so they are not susceptible to nitrite poisoning from excessive intake of nitrates.

However, they are highly susceptible to poisoning from nitrite intake (for instance in moldy hay- termed preformed Nitrites) because they cannot convert the nitrite to ammonia.

Ruminant



Non-ruminant



Sheep vs. cows

Sheep are more efficient at converting nitrite to ammonia, so this may be the reason why they are less susceptible to nitrite poisoning than cattle.

LD_{50} of Nitrate (poor agreement on lethal dose)

Adult Human	NO_3 80-300 mg/kg
Cattle	NO_3 330-620 mg/kg
Sheep	NO_3 308 mg/kg

Factors influencing lethal dose:

- Animal variation
- Route of administration
- Dose
- Duration
- Length of adaptation
- Physical or temporal diet differences

Treatment

Urgent attention is required to confirm the tentative diagnosis and to treat affected animals.

Animals should immediately be removed from suspect material, and be handled as little and as quietly as possible.

Hay or some other low-nitrate herbage should be fed to dilute the nitrate and/or nitrite in the stomach.

Treatment (Cont.)

Affected animals can be treated by intravenous injections of methylene blue, a powdered dye material (15 mg/kg b.w.)

Methylene blue converts the methemoglobin back to oxygen-carrying hemoglobin.

Note: Methylene blue is no longer approved by FDA for use in food-producing animals. Vets will still use it however.

Feeding Forage with Nitrate

NO3-N (%)	NO3-N (ppm)	Feeding level
< 0.10	1000	Safe always
0.1-0.15	1000-1500	Generally safe
0.15-0.20	1500-2000	< 50% Diet
0.20-0.35	2000-3500	< 35% Diet
0.35-0.40	3500-4000	< 25% Diet
> 0.40	> 4000	Potentially toxic

Prevention

The risk of poisoning can be reduced by:

- having feeds and forages analyzed for nitrate when in doubt, such as drought-stressed, small-grain forages;
- not grazing stock on forages that are potentially dangerous;
- observing livestock frequently when put on potentially risky feed;

Prevention

Feed hungry animals on dry hay or mature grass before allowing free access to immature cereal crops;

Prevent hungry livestock from eating recently sprayed weeds;

Prevent hungry animals from over-eating highly fertilized crops.

Prevention

Avoid overgrazing — can result in more stalks being consumed (the stalk contains the most nitrate in the plant). Avoid strip grazing for the same reason.

Avoid grazing high-nitrate pastures or crops for 7 days after periods of rainfall, cloudy days, frosts, or high temperatures that cause wilting.

Time grazing: graze on high-nitrate pastures during sunny afternoons (when the temperature is above 70F) and remove at night. This reduces the amount of high-nitrate forage consumed and helps rumen microbes to adapt.

Prevention

Prevent access to high-risk weeds around barnyards & corrals.

Feed risky material in small amounts, and gradually increasing the amount fed — this applies only to ruminants;

Ensure water does not contain high levels of nitrates.

Do not feed green chop that has heated after cutting.

Never feed moldy hay.

Optional Information on Nitrate Toxicity in next 10 slides

INTERPRETING AND USING NITRATE REPORTS

Results of nitrate analysis may be confusing because of the variation in methods of reporting. Further confusion and questions exist because of the absence of a specific guideline on what levels of nitrate can be considered as safe.

Table 1. Converting one form of nitrate to another.		
Reported as	To convert reported data to one of these multiply by:	
	NO_3-N	NO_3
Nitrate-nitrogen (NO_3-N)	---	4.4
Nitrate (NO_3)	.23	---

(1) Example: 0.1% NO_3-N is equal to 0.44% NO_3 (.1 x 4.4). 0.44% NO_3 is equal to 0.1% NO_3-N (.44 x .23)

Converting % to ppm - Move decimal point four places to the right.
Example: 0.1 percent equals 1000 ppm.

Converting ppm to % - Move decimal point four places to the left.
Example: 750 ppm equals .075 percent.

Field Test for Nitrates

Diphenylamine test: 3 drops reagent mixed with suspect fluid (urine, blood, macerated plant tissue)

Blue color indicates nitrate reaction

False positives can occur

Diphenylamine reagent

Place diphenylamine (0.5 g) in a graduated cylinder or flask containing 20 ml of distilled water.

While stirring, slowly add 88 ml of concentrated sulfuric acid (H_2SO_4). This will dissolve the diphenylamine and generate heat.

Allow the reagent to cool, then place it in a clean dropper bottle. Since the reagent contains sulfuric acid, it must be handled with caution.

Nitrates in Water

"blue baby syndrome" – formula from N-water

Water from deep wells fed by soil water from highly fertile soils.

Fluids draining from silos containing materials rich in nitrates.

Water contaminated by fertilizer, animal wastes or decaying organic matter may also be a source of toxic levels. Marginally toxic levels of nitrate in water, combined with marginally toxic levels of nitrate in feed, can also lead to poisoning.

Interesting factoid

Methemoglobin reductase, in red blood cells, is responsible for maintaining hemoglobin heme iron in its functional 2+ state. Because the iron is in close contact with its reactive load of molecular oxygen, "accidental" oxidation of the iron occurs continuously. That damage is reversed continuously by methemoglobin reductase.

Blue people: individuals born with a congenital deficiency of methemoglobin reductase. There is a relatively high incidence of this trait among Alaskan Eskimos and among some families in Appalachia. These individuals may go through life with as much as half of their total hemoglobin in the form of methemoglobin. Typically, they are more blue than sick. They can compensate for the defect by making more red blood cells than normal individuals.

Nitrate vs. other anoxias

Nitrite	Blood brown	Methemoglobinemia
Cyanide	Blood cherry red	Slight MetHB; oxyhemeglobin
CO2	Blood blue	No oxygen attached to heme (CO2 only)
CO	Blood bright red	Carboxyhemeglobin (1 unit) & tightly bound Oxygen (3 units)

When carbon monoxide is inhaled, it takes the place of oxygen in hemoglobin, the red blood pigment that normally carries oxygen to all parts of the body. Because carbon monoxide binds to hemoglobin several hundred times more strongly than oxygen, its effects are cumulative and long-lasting, causing oxygen starvation throughout the body.

[Warning: Slight digression here] CO poisoning and Haunted houses

Symptoms of carbon monoxide poisoning include listlessness, depression, dementia, emotional disturbances, and hallucinations. Many of the phenomena generally associated with haunted houses, including strange visions and sounds, feelings of dread, illness, and the sudden, apparently inexplicable death of all the occupants, can often be attributed to carbon monoxide poisoning.

Nitrates can help counteract cyanide poisoning

Methemoglobin has an affinity for cyanide molecules, thus low levels of nitrite poisoning can alleviate some aspects of cyanide poisoning.