METHYLENE BLUE (Veterinary—Systemic)

There are no veterinary-labeled commercial products in the United States or Canada.

Category: Antimethemoglobinemic.

Indications

Note: Methylene blue is not specifically approved for veterinary use. In other USP information monographs, the [EL]US and [EL]CAN designations refer to uses that are not included in U.S. and Canadian product labeling; however, in this monograph they reflect the lack of veterinary product availability in the countries indicated. See also the *Regulatory Considerations* section below in this monograph.

Classification as *Accepted*, *Potentially effective*, or *Unaccepted* is an evaluation of reasonable use that considers clinical circumstances, including the availability of other therapies. The quality of evidence reviewed for an indication is shown by the evidence rating.

General considerations

Methemoglobin occurs as the result of oxidative damage to hemoglobin. Blood containing high concentrations of methemoglobin is chocolate brown in color and cannot transport oxygen efficiently. Minor amounts of methemoglobin in the blood may be reduced back to active hemoglobin by innate enzyme systems. However, when exposure to a significant amount of an oxidizing toxicant occurs and the percentage of functioning hemoglobin in the blood drops, oxygen transport can be disabled to the point of hypoxia, suffocation, and death.

Species vary in their ability to protect hemoglobin from oxidation, depending on the structure of their hemoglobin, the effectiveness of metabolic pathways to reverse oxidation, and vulnerability to particular toxicants. In cats and human beings, methemoglobin may comprise up to 2% of hemoglobin in the blood at any one time under normal circumstances. Heinz bodies are caused by oxidation of hemoglobin at a different site on the molecule than that oxidized in methemoglobin.{R-15} In cats and dogs, administration of methylene blue to treat methemoglobinemia can cause oxidative damage to erythrocytes, including Heinz body formation, limiting the dose that can be used therapeutically.

In ruminants, methemoglobinemia is most commonly reported from exposure to nitrate, nitrite, or chlorate. Sources include plants (accumulation from the soil) or water (contamination from soil, fertilizers, or treated whey). Nitrate is reduced to the more toxic nitrite in the rumen; therapy may include intervention to slow reduction of nitrate by rumen microorganisms. Chlorate exposure may come from plants or water contaminated with herbicide.{R-14; 34}

For cats in particular, but also dogs, there are household sources of methemoglobin-producing toxicants, including naphthalene (mothballs) and acetaminophen (also hepatotoxic in cats); however, methylene blue is not the treatment of choice for acetaminophen poisoning (see below).{R-10; 14} Local anesthetics, such as benzocaine, can also cause significant methemoglobinemia, if not carefully administered.{R-12; 14}

Cattle, goats, and *sheep*

Accepted

[EL]US,CAN Methemoglobinemia (treatment)[EL]—Methylene blue may be used in the emergency treatment of methemoglobinemia in ruminants (Evidence rating: B-2,3).{R-1-6}

Cats

Potentially effective

[EL]US,CAN Methemoglobinemia (treatment)[EL]—Methylene blue may be used in the emergency treatment of methemoglobinemia in cats; however, the risk of inducing hemolytic anemia should be balanced with the clinical need to treat. Careful dosing, concurrent fluid therapy, and post-treatment monitoring is recommended (Evidence rating: B-2,3).{R-10-13; 28}

For acetaminophen poisoning in cats, acetylcysteine is the treatment of choice. In some cats, possibly

Evidence ratings

Evidence Quality	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Insufficient evidence to support a recommendation for use
D	Moderate evidence to support a recommendation against use
E	Good evidence to support a recommendation against use

Evidence Type	
1	Species-specific evidence from at least one large randomized and controlled trial (RCT) or multiple small RCTs
2	Species-specific evidence from a small RCT, disease models, large case studies, pharmacokinetic studies using surrogate endpoints, or evidence from well-designed trials in a different species that is considered appropriate for comparison
3	Dramatic results from either well-designed, species-specific trials without controls, controlled trials without randomization, or small case studies
4	Pharmacokinetic studies without surrogate endpoints or well designed pharmacodynamic studies in healthy animals
5	*In vitro* studies
6	Opinions of respected authorities on the basis of clinical experience or reports of expert committees

male cats in particular, methylene blue can exacerbate acetaminophen-induced methemoglobinemia.{R-10}

Dogs

Potentially effective

EL US,CAN Methemoglobinemia (treatment) EL—Acute methemoglobinemia in dogs may warrant the administration of methylene blue; however, such use should take into consideration the adverse effects (Evidence rating: B-3,6).

Methylene blue causes Heinz body formation in cat and dog erythrocytes; in addition, another visible oxidative change, described as "blistering," appears in dog erythrocytes. The occurrence and severity of anemia is dose-dependent; however, debilitated animals appear to be more susceptible. If methylene blue is administered, careful dosing, concurrent fluid therapy, and post-treatment monitoring for at least several days are recommended.{R-20-22; 31; 40-42}

Horses

Note: There are no equine-specific *in vivo* studies of the efficacy or safety of methylene blue in the treatment of methemoglobinemia in horses. A single *in vitro* study did not demonstrate an effect on reduction of methemoglobin in equine erythrocytes (Evidence rating: C-6).{R-45} However, in the face of acute methemoglobinemia in horses and no effective alternative treatment, the administration of methylene blue may be warranted, based on evidence of efficacy in other species.

Regulatory Considerations

U.S. and Canada—

There are no commercial veterinary methylene blue products for systemic use. There are commercial human products that may be appropriate for use in some species. Because treatment of methemoglobinemia in large animals or in groups of animals may require significantly more medication than is practical with human products, methylene blue injection may need to be compounded for emergency use. Methylene blue is included in an Appendix A "List of bulk drug substances for compounding and subsequent use in animals to which the Food and Drug Administration Center for Veterinary Medicine would not ordinarily object (CPG 7125.40)." {R-38} However, there are concerns about potential carcinogenicity and the need for extended withdrawal times.{R-32; 33} See the *Dosages* section in this monograph for more information about residue withdrawal intervals.

In the United States, refer to the Animal Medicinal Drug Use Clarification Act,{R-36} Food and Drug Administration regulations pertaining to compounding (CFR 21 Part 530.13),{R-37} and the current United States Food and Drug Administration's Compliance Policy Guide on *Compounding of Drugs for Use in Animals*.{R-38} In Canada, refer to the Health Canada Health Products and Food Branch's *Policy on Manufacturing and Compounding Drug Products in Canada*.{R-39}

Chemistry

Chemical group: Thiazine dye of the quinonoimine group.{R-6}

Chemical name: Phenothiazin-5-ium, 3,7-bis(dimethylamino)-, chloride, trihydrate{R-23}

Molecular formula: $C_{16}H_{18}ClN_3S \cdot 3H_2O$.{R-23}

Molecular weight: 373.90.{R-23}

Description: Methylene Blue USP—Dark green crystals or crystalline powder having a bronze-like luster. Is odorless or practically so, and is stable in air.{R-24}

pKa: Methylene blue and leucomethylene blue—Less than 1. Some authors have reported the pKa to be in the 0 to –1 range.{R-4; 5; 17}

Solubility: Methylene Blue USP—Its solutions in water and in alcohol are deep blue in color. Soluble in water and in chloroform; sparingly soluble in alcohol.{R-24}

Pharmacology/Pharmacokinetics

Note: See also *Table I* at the end of this monograph.

Mechanism of action/Effect: Methylene blue acts as a cofactor to accelerate the conversion of methemoglobin to hemoglobin in erythrocytes. Methylene blue is an electron carrier and is quickly reduced to leucomethylene blue in the body by combining with reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of NADPH reductase. Leucomethylene blue is then able to rapidly transfer the added electron to reduce methemoglobin to hemoblobin. This reaction oxidizes leucomethylene blue to methylene blue, which can again enter the cycle of reduction and oxidation until it is biotransformed or eliminated.{R-2; 9; 25}

Other actions or effects:

Paradoxically, at higher concentrations, methylene blue can cause some conversion of hemoglobin back to methemoglobin; however, this effect is more evident

in *in vitro* studies and may be minimal in intact erythrocytes.{R-6; 7; 25; 27} Conversion has been shown to be minor in sheep and could not be demonstrated in dogs or rats given extremely high doses of methylene blue.{R-3; 6}

Absorption:

Intramammary—*Goats:* Methylene blue is rapidly absorbed systemically when administered into the mammary gland.{R-5}

Oral—

Dogs: About 60 to 70% of an oral dose is absorbed.{R-16}

Human data: About 74% of an oral dose is absorbed.{R-16}

Distribution: Methylene blue appears to be rapidly distributed into tissues. When rats were administered a dose of 2 to 25 mg per kg of body weight (mg/kg) and euthanized 3 minutes later, 30% of the dose was already found in the four organs surveyed: lungs, liver, kidneys, and heart.{R-18}

Protein binding:

Cattle—Binding to serum and body organs, measured by equilibrium dialysis, was found to be 40 to 60%.{R-4}

Rabbits—71 to 77%.{R-19}

Biotransformation: In mammals, methylene blue is rapidly reduced to leucomethylene blue, a form that is colorless and can be oxidized back to methylene blue.{R-8}

Cattle—Metabolites have been identified by analysis of milk from lactating cattle given methylene blue. They include different stages of demethylated drug (azures and thionin) and a thionin-protein conjugate.{R-8}

Elimination:

Cattle and *goats*—Less than 1% of a dose of methylene blue is eliminated intact in the feces and <2% in urine.{R-4}

Small amounts of drug were found in kidneys three days after a methylene blue dose of 10 mg/kg was given but no residues were found in tissues or fluid at 6 and 9 days after treatment (limit of assay was 3 to 6 parts per billion in biological fluids).{R-4}

Dogs—

Intravenous administration: Of a 15-mg/kg dose administered to dogs, 7% was eliminated in the urine and 20% in the feces. The methylene blue in the urine was 93% leucomethylene blue and in the feces was 24% leucomethylene blue.{R-16}

Oral administration: Of a 15-mg/kg dose administered, 2 to 4% was eliminated in the urine and 44% in the feces.{R-16; 17} The methylene blue in the urine was 97% leucomethylene blue and in the feces was 44% leucomethylene blue.{R-16}

Sheep—Only 6% of the administered dose of methylene blue is eliminated intact in the urine within the first 4 hours.{R-6}

Human data—An average of 74% of the administered dose of methylene blue is eliminated in the urine; 78% of this is in the reduced leucomethylene blue form.{R-6}

Precautions to Consider

Carcinogenicity

Methylene blue is considered mutagenic and a suspected carcinogen.{R-4; 33}

Species sensitivity

Cats and *dogs*—Feline hemoglobin is very susceptible to formation of Heinz bodies with exposure to methylene blue.{R-10} Dogs will also develop Heinz bodies and other oxidative damage to erythrocytes.{R-21}

Reproduction/Pregnancy

Human data: No human or animal studies are available. Human products have been given Pregnancy Category C; safety for use in pregnancy has not been established.{R-25}

Methylene blue was associated with jejunal atresia when administered intra-amniotically during obstetric procedures in women and has been considered a teratogen.{R-26}

Lactation

Cattle and *goats:* Although methylene blue would be expected to be poorly soluble in lipids, it and/or its reduced form, leucomethylene blue, is distributed into milk at concentrations higher than in plasma.{R-4} The milk to blood concentration ratio has averaged 4.8 to 6.37 during an intravenous infusion.{R-5}

A variety of metabolites are also distributed into milk. The predominant is believed to be thionin, found at 27 parts per billion 72 hours after systemic administration of methylene blue.{R-8}

Pediatrics

The safety of methylene blue in immature animals is unknown.

Human data: Up to four months of age, human infants have a decreased capacity to reduce methemoglobin

to hemoglobin, making them more susceptible to potential adverse effects associated with high doses of methylene blue.{R-25}

Drug interactions and/or related problems

The following drug interactions and/or related problems have been selected on the basis of their potential clinical significance (possible mechanism in parentheses where appropriate)—not necessarily inclusive (» = major clinical significance):

Note: No significant drug interactions have been reported in association with methylene blue administration in animals.

Laboratory value alterations

The following have been selected on the basis of their potential clinical significance (possible effect in parentheses where appropriate)—not necessarily inclusive (» = major clinical significance):

Note: No significant laboratory value alterations have been reported in association with methylene blue administration in animals.

Medical considerations/Contraindications

The medical considerations/contraindications included have been selected on the basis of their potential clinical significance (reasons given in parentheses where appropriate)—not necessarily inclusive (» = major clinical significance).

Except under special circumstances, this medication should not be used when the following medical problems exist:

All species

Hypersensitivity to methylene blue

Cats and *dogs*

Renal failure or

Risk factors for acute renal failure, including

Acidosis

Dehydration

Hypercalcemia, chronic

Hypoxia

(Because dose-dependent erythrocyte oxidation occurs with even the low doses recommended for therapeutic use of methylene blue in cats and dogs, Heinz body formation or other oxidative changes may lead to erythrocyte destruction, increasing the risk of hemoglobinuric nephrosis){R-20-22}

Risk-benefit should be considered when the following medical problems exist:

Cats and *dogs*

Anemia, severe, or

Hemolytic anemia, history of

(Because of erythrocyte oxidation caused by methylene blue, the presence of pre-existing severe anemia should be considered in the decision to treat and the dosage administered){R-20-22}

Patient monitoring

The following may be especially important in patient monitoring (other tests may be warranted in some patients, depending on condition; » = major clinical significance):

Complete blood count and

Hematocrit and

Reticulocyte count

(Monitoring red cells for oxidative changes and/or hemolytic anemia is important in cats, dogs, horses, and other susceptible species administered methylene blue. In cats and dogs, anemia may take 3 to 4 days to appear.){R-11; 21; 22; 28}

Methemoglobinemia

(When it is necessary to administer high or repeated doses, methemoglobin determination can be useful. Once clinical signs appear, about 40 to 50% of hemoglobin has been oxidized to methemoglobin; 70 to 80% is generally lethal.){R-3; 11; 35}

Mucous membrane color

(If cyanosis is present, mucous membrane color may be used as an indicator of treatment response. Severe methemoglobinemia can cause a characteristic, chocolate-colored darkening of the mucous membrances.)

(Researchers have described a pseudocyanosis in some dogs from methylene blue coloration during intravenous infusion of a 3-mg/kg dose.){R-21; 22}

Side/Adverse Effects

The following side/adverse effects have been selected on the basis of their potential clinical significance (possible signs in parentheses where appropriate)—not necessarily inclusive:

Those indicating need for medical attention

Incidence unknown

Cats

Heinz body production{R-11; 13}

Note: The development of hemolytic anemia in response to methylene blue administration is a dose-dependent effect that may take 3 to 4 days to appear. Heinz bodies will more rapidly appear in feline erythrocytes than in human or canine erythrocytes when exposed *in vitro* to methylene blue; therefore, careful dosing and monitoring post-treatment is recommended.{R-29} Cats appear to be slower than other species to remove the oxidized red cells from

circulation. In cats treated with a single 1.5-mg/kg dose, Heinz bodies can increase from the normal 0 to 2% of cells to up to 9%, without producing anemia. When healthy cats were given two 1.5-mg/kg doses of methylene blue 4 hours apart, Heinz bodies appeared in 21 to 50% of erythrocytes, without producing anemia, even when the cats were pretreated with nitrite to induce methemoglobinemia.[R-11]

Dogs

Oxidative changes in erythrocytes, including Heinz body production[R-21]

Note: The development of anemia in response to methylene blue administration is a dose-dependent effect and may take 3 to 4 days to appear. Heinz body formation is not typically seen in the erythrocytes of healthy dogs. It has been reported in some dogs with a methylene blue dosage as low as 1 mg/kg. Another dose-dependent oxidative change seen in canine red cells with a dose of 1 mg/kg has been described as cell "blistering," an effect reported in association with decreasing packed cell volume.[R-21]

Those indicating need for medical attention only if they continue or are bothersome

Incidence unknown

Cats and *dogs*

Blue to green staining of urine and feces[R-13; 21; 22]

Note: Visible dye in urine and feces may depend on dose and on variations in reduction and/or metabolism of methylene blue. An animal's environment (flooring, etc.) may also be stained by contact with the stained urine or feces.

Overdose

For more information in cases of overdose or unintentional ingestion, **contact the American Society for the Prevention of Cruelty to Animals (ASPCA) National Animal Poison Control Center** (888-426-4435 or 900-443-0000; a fee may be required for consultation) **and/or the drug manufacturer**.

Lethal dose

LD_{50}—*Sheep:* 42.3 mg/kg.[R-6]

The lethal dose is expected to be significantly lower in cats and dogs, as 5 mg/kg has produced severe anemia in dogs.[R-21]

Clinical effects of overdose

The following effects have been selected on the basis of their potential clinical significance (possible signs in parentheses where appropriate)—not necessarily inclusive:

Cats[R-13]

Reported with an oral dose of about 3.5 mg/kg a day for eleven to fourteen days or 5.4 mg/kg every eight hours for seven doses:

Anemia, hemolytic, severe—within three to four days; ***blue staining of urine and feces***

Dogs[R-21]

With a single intravenous dose between 3 and 5 mg/kg:

Anemia, hemolytic, severe—within three to four days

Treatment of overdose

Treatment may include the following:

- Monitoring for and treatment of hemolytic anemia
- Supportive therapy

Client Consultation

In providing consultation, consider emphasizing the following selected information:

Familiarizing clients with the need for monitoring for adverse effects for several days after emergency treatment

General Dosing Information

The amount of methylene blue required to counteract toxicosis depends on the amount of exposure to methemoglobin inducers. Ideally, it should be administered to effect in order to reduce sufficient methemoglobin to hemoglobin to prevent asphyxiation.

In both cats and dogs, oxidative changes in erythrocytes exposed to methylene blue limit the dose that can be administered to treat methemoglobinemia.

Ruminants appear to be less sensitive to adverse effects of methylene blue, allowing it to be given to effect, with some limitations.[R-6] Concerns about carcinogenicity make it prudent to consider withdrawal intervals when deciding to use this medication. When treating toxicosis, clinicians should take into consideration the possibility of ongoing metabolism of nitrate in the rumen prolonging exposure to nitrite.

Dosing and Dosage Forms

Note: Methylene blue is not specifically approved for veterinary use. In other USP information monographs the $^{EL US}$ and $^{EL CAN}$ designations indicate uses that are not included in U.S. and Canadian product labeling; however, in this section they reflect the lack of veterinary products and, therefore, product labeling.

Until dosing studies are performed using a methylene blue preparation made by an accepted, standard compounding formula, the most effective dose may depend on how the drug preparation is compounded. Ranges are given reflecting the information available at this time.

DOSAGES

EL US,CAN ***Cats***—

For *Methylene Blue Injection USP* or, if necessary, *Methylene Blue Injection, Veterinary*

Methemoglobinemia: Intravenous, 1.5 mg per kg of body weight, as a single dose, administered slowly.{R-11-12; 20-22; 28; 40-42}

Note: This dose can be rapidly effective but will produce a certain amount of oxidative damage to erythrocytes (Heinz body formation) that is typically subclinical. The risk of red cell damage and subsequent anemia increases with repeated or higher dosing. Using caution when dosing, and monitoring animals for anemia for up to 3 to 4 days after therapy, are recommended.EL

EL US,CAN ***Dogs***—

For *Methylene Blue Injection USP* or, if necessary, *Methylene Blue Injection, Veterinary*

Methemoglobinemia: Intravenous, 1.5 mg per kg of body weight, as a single dose, administered slowly.{R-11-12; 20-22; 28; 31; 40-42}

Note: There is some evidence that this dose can cause sufficient oxidative red cell damage to make it prudent to monitor patients for three to four days. A dose of 5 mg/kg has produced anemia requiring blood transfusion in dogs.{R-20-22; 40}EL

EL US,CAN ***Cattle, goats,*** and ***sheep***—

For *Methylene Blue Injection, Veterinary*

Methemoglobinemia: Intravenous, 4 to 10 mg per kg of body weight, given to effect.{R-1; 3; 6} The action of methylene blue is typically rapid, within 15 minutes, and the low end of the dosage range may be repeated to titrate to clinical response. The high dose may be repeated at six to eight hour intervals, as necessary.{R-9} For severe poisoning, 15 to 20 mg or more per kg of body weight might be administered initially.{R-3; 6}

Because of the potential for ongoing ruminal conversion of nitrates to nitrite, monitoring and treatment over hours may be necessary.EL

Extra-label withdrawal recommendation—Due to the potential carcinogenicity of methylene blue, in 2000 the Food and Drug Administration Center for Veterinary Medicine (FDA CVM) recommended a conservative withdrawal period of 180 days for methylene blue administered to any food-producing species, regardless of dose, and strongly recommended that it not be administered to lactating dairy cattle unless the extended withdrawal is met.{R-33}

Cattle: The Food Animal Residue Avoidance Databank (FARAD) in the United States and Canada states that if methylene blue is administered to cattle at a dose of 10 mg per kg of body weight, evidence has been compiled that suggests a milk withholding interval of 96 hours and a meat withdrawal interval of 14 days would be sufficient to avoid residues.{R-32; 43; 44} There is no available information to make recommendations for withdrawal intervals when methylene blue is administered to cattle at a dose higher than 10 mg per kg of body weight or when given to other ruminants at any dose.{R-32; 43; 44}

DOSAGE FORMS

Parenteral

METHYLENE BLUE INJECTION USP

Strength(s) usually available:

United States—

Veterinary-labeled products:

Not commercially available.

Human-labeled products:{R-27}

10 mg/mL (Rx) [GENERIC].

Canada—

Veterinary-labeled products:

Not commercially available.

Human-labeled products:

10 mg/mL (Rx) [GENERIC].

Note: The above products are available in sizes of 1, 5, or 10 mL vial or ampule.

Packaging and storage: Store below 40 ºC (104 ºF), preferably between 15 and 30 ºC (59 and 86 ºF), unless otherwise specified by manufacturer. Protect from light.

USP requirements: See the *Methylene Blue Injection USP* monograph in the *USP-NF*.{R-24}

METHYLENE BLUE INJECTION, VETERINARY

Note: A maximum intravenous dose of 30 mg of methylene blue per kg of body weight is being used to set *USP-NF* endotoxin limits for this dosage form.

Strength(s) usually available: Methylene blue injection is not available as a commercial product packaged in the strength and volume necessary for treatment of large animals in the United States or Canada. Therefore, it must be compounded for veterinary use. Utilizing the services of a qualified compounding pharmacist to formulate this dosage form is recommended.

Packaging and storage: Pending.

USP requirements: Pending.

Table I. Pharmacology/Pharmacokinetics—Intravenous Administration*

Species	$T_{1/2}$ (hours)	Vd (L/kg)	Clearance (mL/min/kg)
Dogs{R-18}		0.222/0.876†	
Sheep{R-6}	1.7 ± 0.3	Vd_{area} = 0.404 ± 0.104	2.8 ± 0.57
Sheep, with induced methemoglobinemia{R-6}	1.8 ± 0.5	Vd_{area} = 0.613 ± 0.121	4.60 ± 0.76

*$T_{1/2}$ = half-life of elimination, Vd = volume of distribution
†Based on nonlinear (one fluid-one tissue) model analysis and classical linear two-compartment open model analysis, respectively.

Developed: 08/31/08

References

1.Yeruham I, Shlosberg A, Liberboim M. Nitrate toxicosis in beef and dairy cattle herds due to contamination of drinking water and whey. Vet Human Tox 1997 Oct; 39(5): 296-8.

2.Van Dijk S, Lobsteyn AJ, Wensing T, et al. Treatment of nitrate intoxication in a cow. Vet Rec 1983 Mar 19; 112: 272-4.

3. Burrows GE. Methylene blue or tolonium chloride antagonism of sodium nitrite induced methemoglobinemia. J Vet Pharm Ther 1979; 2: 81-6.

4. Ziv G, Heavner JE, Kawalek J. Pharmacokinetic and depletion studies of methylene blue in ruminants. Les Colloques de l'INRA 1982; 8: 491-2.

5. Ziv G, Heavner JE. Permeability of the blood-milk barrier to methylene blue in cows and goats. J Vet Pharmacol Ther 1984; 7: 55-59.

6. Burrows GE. Methylene blue: effects and disposition in sheep. J Vet Pharmacol Ther 1984; 7: 225-31.

7. Smith RP, Thron CD. Hemoglobin, methylene blue and oxygen interactions in human red cells. J Pharmacol Exp Ther 1972 Dec; 183(3): 549-58.

8. Roybal JE, Pfenning AP, Turnipseed SB, et al. Dye residues in foods of animal origin. In: Veterinary drug residues : food safety. Washington, DC : American Chemical Society. 1996, p. 169-183.

9. Nitrates, nitrites and related problems. In: Osweiler GD, Carson TL, Buck WB, et al. Clinical and diagnostic veterinary toxicology, 3rd ed. Dubuque, Iowa: Kendall/Hunt Publishing Company. 1973. p. 460-7.

10. Rumbeiha WK, Lin Y, Oehme FW. Comparison of N-acetylcysteine and methylene blue, alone or in combination, for treatment of acetaminophen toxicosis in cats. Am J Vet Res 1995; 56(11): 1529-33.

11. Rumbeiha WK, Oehme FW. Methylene blue can be used to treat methemoglobinemia in cats without inducing Heinz body hemolytic anemia. Vet Hum Toxicol 1992 Apr; 34(2): 120-2.

12. Wilkie DA, Kirby R. Methemoglobinemia associated with dermal application of benzocaine cream in a cat. J Am Vet Med Assoc 1988 Jan 1; 192(1): 84-6.

13. Schechter RD, Schalm OW, Kaneko JJ. Heinz body hemolytic anemia associated with the use of urinary antiseptics containing methylene blue in the cat. J Am Vet Med Assoc 1973 Jan 1; 162(1): 37-44.

14. Beasley V. Methemoglobin producers. In: Beasley V, editor. Veterinary Toxicology. Ithaca, New York: International Veterinary Information Service. Available at www.ivis.org/advances/Beasley/toc.asp. Accessed on January 29, 2008.

15. Houston DM, Myers SL. A review of Heinz-body anemia in the dog induced by toxins. Vet Hum Toxicol 1993 Apr; 35(2): 158-61.

16. Watanabe J, Fujita R. Elimination of methylene blue in dogs after oral or intravenous administration. Chem Pharm Bull 1977 Oct; 25(10): 2561-7.

17. DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs II: Methylene blue—absorption, metabolism, and excretion in man and dog after oral administration. J Pharm Sci 1972 Jul; 61(7): 1086-90.

18. DiSanto AR, Wagner JG. Pharmacokinetics of highly ionized drugs III: methylene blue—blood levels in the dog and tissue levels in the rat

following intravenous administration. J Pharm Sci 1972 Jul; 61(7): 1090-4.
19. Kozaki A, Watanabe J. Dose dependency of apparent volumes of distribution for methylene blue in rabbits. J Pharmacobiodyn 1981; 4(1): 49-57.
20. Fingeroth JM, Smeak DD. Intravenous methylene blue infusion for intraoperative indentification of parathyroid gland tumors in dogs. Part III: clinical trials and results in three dogs. J Am Anim Hosp Assoc 1988 Mar/Apr; 24: 175-82.
21. Fingeroth JM, Smeak DD, Jacobs RM. Intravenous methylene blue infusion for intraoperative identification of parathyroid gland and pancreatic islet-cell tumors in dogs. Part I. Experimental determination of dose-related staining efficacy and toxicity. J Am Anim Hosp Assoc 1988 Mar/Apr; 24: 165-73.
22. Fingeroth JM, Smeak DD. Intravenous methylene blue infusion for intraoperative indentification of pancreatic islet-cell tumors in dogs. Part II: clinical trials and results in four dogs. J Am Anim Hosp Assoc 1988 Mar/Apr; 24: 175-82.
23. USP dictionary of USAN and international drug names, 43rd edition. Rockville, MD: The United States Pharmacopeial Convention Inc. 2007. Available at www.uspusan.com. Accessed on February 18, 2007.
24. The United States Pharmacopeia. The national formulary. USP 30th revision (August 1, 2007). NF 25th ed. (August 1, 2007). Rockville, MD: The United States Pharmacopeial Convention Inc; 2007. Available at www.uspnf.com. Accessed on February 18, 2007.
25. Methylene Blue (Systemic). In: USP DI Drug information for the healthcare professional. Volume I. Greenwood Village, CO: MICROMEDEX, Inc.; 1998.
26. Bailey B. Are there teratogenic risks associated with antidotes used in the acute management of poisoned pregnant women? Birth Defects Res A Clin Mol Teratol 2003; 67: 133-40.
27. Methylene Blue Injection USP 1% package labeling (American Regent—US), Rev 1/03. Available at www.americanregent.com. Accessed on January 1, 2008.
28. Harvey JW, Keitt AL. Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinemia. Br J Haematol 1983; 54: 29-41.
29. Harvey JW, Kaneko JJ. Interactions between methylene blue and erythrocytes of several mammalian species, in vitro. Proc Soc Exp Biol Med 1974; 147(1): 245-9.
30. Stossel TP, Jennings RB. Failure of methylene blue to produce methemoglobinemia in vivo. Am J Clin Path 1966; 45(5): 600-4.
31. Harvey JW, King RR, Berry CR, et al. Methaemoglobin reductase deficiency in dogs. Comp Hematol Int 1991; 1: 55-9.
32. Haskell SR, Payne M, Webb A, et al. Antidotes in food animal practice. J Am Vet Med Assoc 2005 Mar 15; 226(6): 884-7.
33. Post LO, Keller WC. Current status of food animal antidotes. Vet Clin N Am Food Anim Pract 2000 Nov; 16(3): 445-53.
34. Booth NH, McDonald LE, editors. Veterinary Pharmacology and Therapeutics, 6th ed. Ames, Iowa: Iowa State University Press, 1988. p. 1036-8.
35. Davis LE. Nitrate intoxication. J Am Vet Med Assoc 1980 Jul 1; 177(1): 82-3.
36. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Public Law 103-396. Available at http://www.fda.gov/cvm/s340.htm. Accessed February 28, 2008.
37. Office of the Federal Register. Code of Federal Regulations. 21 Part 530.13. US Government Printing Office. Available at www.access.gpo.gov/nara/cfr/waisidx_07/21cfr530_07.html. Accessed on February 28, 2008.
38. Compounding of drugs for use in animals (CPG 7125.40). In: Compliance policy guides manual. Section 608.400. United States Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine. July, 2003. Available at http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg608-400.html. Accessed on February 28, 2008.
39. Policy on manufacturing and compounding drug products in Canada. Health Canada Health Products and Food Branch. November 17, 2006. Available at www.hc-sc.gc.ca. Accessed on February 28, 2008.
40. Smeak DD, Fingeroth JM, Bilbrey SA. Intravenous methylene blue as a specific stain for primary and metastatic insulinoma in a dog. J Am Anim Hosp Assoc 1988; 24(5): 478-80.
41. Osuna DJ, Armstrong PJ, Duncan DE, et al. Acute renal failure after methylene blue infusion in a dog. J Am Anim Hosp Assoc 1990 Jul/Aug; 26: 410-2.
42. Fingeroth JM, Smeak DD. Methylene blue infusion [Letter]. J Am Anim Hosp Assoc 1991; 27(3): 259.
43. Communication with the Food Animal Residue Avoidance Databank, March 10, 2008.
44. Communication with the Canadian gFARAD, March 10, 2008.
45. Medeiros LO, Nurmberger R, Medeiros LF. The special behavior of equine erythrocytes connected with the methemoglobin regulation. Comp Biochem Physiol 1984; 78B(4): 869-71.

Methylene blue in the treatment of methemoglobinemia in cattle, goats, and sheep.

Revision date: February 28, 2008

Back to the indication.

The following tables are not intended to be in-depth reviews of each article. Instead, they are brief overviews/reviews of the studies, assuming reviewers are already familiar with the cited references. If you would like additional information about these studies, please contact veterinary@usp.org.

Study 1 of 6: Yeruham I, Shlosberg A, Liberboim M. Nitrate toxicosis in beef and dairy cattle herds due to contamination of drinking water and whey. Veterinary and Human Toxicology 1997 Oct; 39(5): 296-8.

Design	**Methods:**	**Comments:**
• Case report N = 95 with toxicosis	• Water contamination—Herd 1 (107 head) and herd 2 (350) cross-breed beef cattle were moved to separate new pastures in hot weather with water troughs that also supplied water to local orchards. Within twenty hours, 20 cattle in herd 1 and 11 cattle in herd 2 were found dead. Twenty other cattle in herd 1 developed cyanosis, ataxia, muscle tremors, and, in some, opisthotonus or abortion. Water from the troughs for herd 1 was found to have 8,000 to 8,800 parts per million (ppm) of nitrate and, for herd 2, 965 to 1,005 ppm. • Whey contamination—Herd 3 (1,000) and herd 4 (800) Israeli-Holstein dairy cattle were fed a total mixed ration with mineral/vitamin premix. Fresh whey supplemented this diet daily for 360 heifers in herd 3 and 140 pregnant cows in herd 4. Several hours after whey was delivered one day, 75 heifers became sick and 17 of those affected died. The whey was found to have 2,200 to 2,800 ppm nitrate. In herd 4, 26 cows aborted over 6 days. This whey was found to have 400 to 800 ppm of nitrate. **Dose:** • Intravenous methylene blue, 50 mL of a 4% solution. The dose was repeated in animals that did not respond within 15 to 20 minutes. **Results:** • Poisoning was diagnosed by clinical signs and confirmed by nitrate analysis of water and whey, response to treatment, and postmortem findings, which included characteristic unclotted chocolate-colored blood and positive aqueous fluid nitrate analysis. • Once the sources of nitrate in the whey and water were identified and removed, no new cases occurred. Animals responded to treatment, although 5 cows in herd 1 and 15 in herd 3 were in poor condition for months. **Conclusions:** • Safe levels of nitrate in drinking water may be up to about 500 ppm. In herd 4, abortion was seen when other clinical illness was not reported.	• This whey toxicosis event was originally reported in Refuah Veterinarit 1980; 37(3): 101-3.

Study 2 of 6: Burrows GE. Methylene blue: effects and disposition in sheep. Journal of Veterinary Pharmacology and Therapeutics 1984; 7: 225-31.

Design • Pharmaco-kinetic and toxicity studies N = 30	**Goal:** To investigate the disposition and safety of methylene blue in sheep. **Methods:** • Mixed-breed, female sheep. Trial 1—Toxic effect and lethal dose of methylene blue: 22 sheep Trial 2—Pharmacokinetics and hematologic effects: 4 sheep received 50 mg of sodium nitrite per kg, followed 20 minutes later by methylene blue. 4 sheep received a saline control, followed by the methylene blue; these sheep also received a second dose of methylene blue, 18 hours after the first. Blood methemoglobin and methylene blue was measured at intervals before and after medication administration. Urine samples were also collected for methylene blue analysis. • One assay measured unchanged methylene blue and the second measured total drug, including leucomethylene blue. **Duration:** 28 days **Dose:** • Trial 1—Intravenous methylene blue, 4.4, 11, 15, 22, 30, or 50 mg/kg • Trial 2—Intravenous methylene blue, 15 mg/kg **Results:** • Trial 1—The production of methemoglobin in response to methylene blue did not rise above 5% of total hemoglobin with doses up to 30 mg/kg. With a dose of 50 mg/kg, methemoglobin reached 11.4%; however, this was a lethal dose in 2 of 4 sheep. LD_{50} was found to be 42.3 mg/kg. • Trial 2— Methylene blue administered alone: $T_{1/2} = 1.7 \pm 0.3$ hours, $Vd_{area} = 0.404 \pm 0.104$ L/kg, Clearance = 2.80 ± 0.57 mL/min/kg Methylene blue administered with sodium nitrite: $T_{1/2} = 1.8 \pm 0.5$ hours, $Vd_{area} = 0.613 \pm 0.121$ L/kg, Clearance = 4.60 ± 0.76 mL/min/kg **Conclusions:** • Methylene blue half-life of elimination was unaffected by nitrite administration; however, the distribution rate, volume of distribution, and clearance were increased. • Doses higher than 4 mg/kg do not appear to be an increased risk of antidote toxicosis. A therapeutic dose of 15 to 20 mg/kg might be considered for severe nitrate poisoning.	

Study 3 of 6: Ziv G, Heavner JE. Permeability of the blood-milk barrier to methylene blue in cows and goats. Journal of Veterinary Pharmacology and Therapeutics 1984; 7: 55-9.

Design	**Goal:** To study, in cattle and goats, the permeability of the blood-milk barrier to methylene blue	**Comments:**
• Pharmaco-kinetic study N = 6 cows, 7 goats	**Methods:** • Six lactating Holstein cattle and seven lactating mixed breed goats near the end of lactation. Four cows had one or more quarters with chronic gram-positive infection. • Assay sensitivity limits for blood and milk were 0.01 and 0.005. • Trial 1—Single intravenous bolus administration: Blood and milk samples were taken periodically from 4 to 48 hours after drug administration. Milk samples continued to be taken from the goats twice a day for four days. Trial 2—Intravenous infusion: Blood and milk samples were taken every half-hour for up to 3.5 hours. Trial 3—Intramammary infusion: Blood and milk samples were taken every 1 to 2 hours for 12 hours and then every twelve hours for 4 days. **Dose:** • Trial 1—Cattle: Intravenous methylene blue, 10 mg/kg • Trial 2—Goats: Intravenous methylene blue bolus, 5 mg/kg, followed by an intravenous infusion of 5 mg/kg, administered over 2 to 3.5 hours • Trial 3—Goats: Intramammary methylene blue, 10% aqueous solution (total dose not given) **Results:** • In cattle, methylene blue was distributed into milk at higher concentration than in blood and was found for up to 36 hours after treatment, while it was no longer detectable in blood after 24 hours. Inflammation in a gland did not appear to affect distribution into milk. • Drug concentrations in milk were similar in cattle and goats. • In the first sample after an intravenous bolus of methylene blue, milk to blood concentration ratio was about 1. During intravenous infusion over 2 to three hours, ratios averaged 4.8 to 6.3. • Intramammary administration of methylene blue results in rapid systemic absorption. **Conclusions:** • The assay used may have been measuring both methylene blue and leucomethylene blue. It's possible that leucomethylene has properties that makes it more likely than methylene blue to passively diffuse across membranes.	• The pharmaco-kinetic study described here appears to be very similar to (the same as?) the one described in Ziv, et al. 1982 (study 5 in this table).

Study 4 of 6: Van Dijk S, Lobsteyn AJ, Wensing T, et al. Treatment of nitrate intoxication in a cow. Veterinary Record 1983 Mar 19; 112: 272-4.

Design	**Goal:** To investigate the efficacy of vitamin C or menadione (vitamin K_3), and to study methylene blue dosing, in the treatment of acute nitrate poisoning in cattle.	**Comments:**
• Open study comparing treatments of induced intoxication N = 1	**Methods:** • A Friesian cow, 10 years old and weighing 600 kg, with a large rumen fistula was given nitrate via tube through the fistula and into the reticulum. • The cow was given 200 grams of potassium nitrate in 1.5 liters of water as a single dose, followed 4 to 6 hours later with the test treatment. Hemoglobin and methemoglobin were measured just before nitrate administration and at intervals during the treatment days. **Dose:** • Intravenous methylene blue, 0.5, 1, and 2 mg/kg • Intravenous ascorbic acid, 1.2 and 20 mg/kg • Intravenous menadione sodium bisulphite, 1 and 2 mg/kg **Results:** • Potassium nitrate consistently produced an increase in blood methemoglobin concentration. Signs of toxicosis appeared (cyanosis, elevated pulse and respiration, muscle trembling, incoordinated gait) and, with no treatment, resolved within a day. • Each dose of methylene blue decreased methemoglobin concentration. Although the 0.5-mg/kg dose was not sufficiently effective, both the 1-mg and 2-mg doses were. Sodium ascorbate did not affect methemoglobin at the dosages given. Menadione only marginally decreased methemoglobin concentration. **Conclusions:** • The minimal dose of methylene blue in the treatment of methemoglobinemia may be 1 mg/kg. • Results suggest that vitamin C and menadione are unsuitable for treatment of methemoglobinemia in cattle.	• Single subject

Study 5 of 6: Ziv G, Heavner JE, Kawalek J. Pharmacokinetic and depletion studies of methylene blue in ruminants. Les Colloques de l'INRA 1982; 8: 491-2.

Design	**Methods:**	
• 1) Pharma-cokinetic study and 2) efficacy study, open with no controls, of cattle with induced nitrite poisoning N = 17 head of cattle, 18 goats	• Pharmacokinetic studies—Blood, urine, feces, and milk were sampled at intervals after drug administration. Animals were euthanized 3, 6, and 9 days after treatment and drug concentrations in tissues were determined. -Single intravenous dose to 6 lactating cows, 6 lactating goats, and 6 steers. -Intravenous infusion for 2 to 3 hours to 6 lactating goats -Intramammary infusion into the glands of 6 lactating goats • Therapeutic study—Five steers were given intraruminal sodium nitrate at a dose of 100 to 200 mg/kg and total hemoglobin was monitored until methemoglobinemia reached 55 to 60% of the total hemoglobin or the animal collapsed, when methylene blue was administered. **Dose:** • Pharmacokinetic studies— Intravenous methylene blue, 10 mg/kg Intravenous infusion (rate not stated) Intramammary methylene blue, 10 mg/kg • Therapeutic study—Intravenous methylene blue, 10 mg/kg **Results:** • Methylene blue was detected in milk, at higher concentrations than blood, for 4 to 32 hours after single dose administration. With the infusion, methylene blue was detected in the milk of goats within 5 minutes; at equilibrium, milk concentrations were 5 to 7 times higher in milk than blood. After intramammary infusion, drug was found in blood from 30 minutes to 12 hours. Serum half-life was 2 to 3 hours. • Of the drug administered, <2% was eliminated in urine and <1% in feces. Small amounts of drug were found in kidneys three days after a 10 mg/kg dose but no residues were found in tissues or fluid at 6 and 9 days after treatment (limit of assay was 3 to 6 parts per billion in biological fluids).{R-4} • Animals recovered clinically from induced nitrate poisoning within 5 to 10 minutes of methylene blue administration. Methemoglobin was reduced to <20% of total hemoglobin by 30 minutes to 2 hours post-treatment. • Binding of methylene blue to serum and body organs was found to be 40 to 60% by equilibrium dialysis. **Conclusions:** • Methylene blue is extensively metabolized in ruminants, crosses the blood-milk barrier by an undefined process, and is distributed beyond the vascular compartment. • Methylene blue kinetics are different during nitrate toxicosis than in healthy animals, but duration of tissue residues does not appear to change.	

Study 6 of 6: Burrows GE. Methylene blue or tolonium chloride antagonism of sodium nitrite induced methemoglobinemia. Journal of Veterinary Pharmacology and Therapeutics 1979; 2: 81-6.

Design • Open, active-controlled study of induced nitrite toxicosis N = 48	**Goal:** To investigate the efficacy of methylene blue or tolonium chloride in the treatment of methemoglobinemia in sheep. **Methods:** • Mature ewes in good health on a dry lot fed mixed alfalfa and grass hay. Sodium nitrite was administered intravenously at various doses, including 6.7, 22, 35, 50, or 100 mg/kg, to induce methemoglobinemia and measure the effect. • Methylene blue or tolonium chloride was administered 30 minutes after a sodium nitrite dose of 50 mg/kg and 10 minutes after a dose of 100 mg/kg. Four ewes received each combination of induction dose and treatment dose. Methemoglobin and hemoglobin were measured at intervals before and during each regimen. **Dose:** • Intravenous methylene blue, 2.2, 4.4, 11, or 22 mg/kg • Intravenous tolonium chloride, 2.2, 4.4, or 6.6 mg/kg **Results:** • The lowest dose of sodium nitrite produced 13% methemoglobin (% of total hemoglobin) within 15 minutes. A dose of 50 mg/kg or more was lethal, with methemoglobin at 70 to 80%. • The 2.2-mg/kg dose of either methylene blue or tolonium chloride was enough to counteract a lethal 50-mg/kg dose of sodium nitrite. The rate of methemoglobin reduction to hemoglobin increased with increasing dose of antidote. • When 100 mg/kg of sodium nitrite was administered, the highest dose of either antidote was insufficient to prevent death, because of the time needed to antagonize the effect (> an hour). • Neither methylene blue or tolonium chloride produced significant amounts of methemoglobin when administered alone; the maximum methemoglobin produced was 6%. **Conclusions:** • The lethal level of methemoglobinemia in sheep appears to be about 75% of hemoglobin. • A tolonium chloride dose of 8.8 mg/kg is more effective than methylene blue at a dose of 22 mg/kg; however, more study needs to be done on the potential toxicity of tolonium chloride. • The data suggest that increasing dosages over those currently used (4 mg/kg) can be effective and are unlikely to produce additional methemoglobin.	

Methylene blue in the treatment of methemoglobinemia in cats.

Revision date: March 1, 2008

Back to the indication.

The following tables are not intended to be in-depth reviews of each article. Instead, they are brief overviews/reviews of the studies, assuming reviewers are already familiar with the cited references. If you would like additional information about these studies, please contact veterinary@usp.org.

Study 1 of 5: Rumbeiha WK, Lin Y, Oehme FW. Comparison of N-acetylcysteine and methylene blue, alone or in combination, for treatment of acetaminophen toxicosis in cats. American Journal of Veterinary Research 1995; 56(11): 1529-33.

Design	**Goal:** To study the efficacy of N-acetylcysteine and methylene blue individually and together in the treatment of methemoglobinemia.	**Limitations:**
• Randomized, controlled study of induced toxicosis N = 30 (15 male and 15 female)	**Methods:** • Healthy cats were given either placebo or acetaminophen, followed 4 hours later by one of several treatments. Tests before and during the study included complete blood count (CBC), blood methemoglobin, blood glutathione, plasma acetaminophen, serum hepatic enzymes, and urine hemoglobin. **Dose:** Each group included 3 male and 3 female cats. Group 1—Water (negative control) Group 2—Oral acetaminophen, 120 mg/kg (positive control) Group 3—Oral acetaminophen, 120 mg/kg, followed in four hours by oral N-acetylcysteine, 280 mg/kg and then N-acetylcysteine, 70 mg/kg every six hours for three days. Group 4—Oral acetaminophen, 120 mg/kg, followed in four hours by intravenous methylene blue, 1.5 mg/kg. Group 5—Oral acetaminophen, 120 mg/kg, followed in four hours by methylene blue and N-acetylcysteine, as described in Groups 3 and 4. **Results:** • There were no significant differences in CBC or hematocrit among any of the groups. Cats that survived appeared healthy at the end of 15 days. • In all cats, acetaminophen induced signs of toxicosis that ranged from mild to fatal. Two cats from Group 4 and one from Group 5 died; all were males. • N-acetylcysteine reduced the half-life of induced methemoglobin from 10.8 hours (Group 2 controls) to 5.0 hours. The longest methemoglobin half-life was seen in cats given both N-acetylcysteine and methylene blue or methylene blue alone. However, looking only at female cats treated with methylene blue or methylene blue/acetylcysteine, the half-life was 2.6 hours and 2.4 hours, respectively; for male cats, it was 33.8 hours and 12.8 hours. **Conclusions:** • The authors concluded the cause of death in 3 male cats was hepatotoxicity, rather than methemoglobinemia. Anemia did not appear to be a factor. • N-acetylcysteine alone appeared to be the best treatment. Male cats appeared to have a significantly longer acetaminophen elimination half-life.	• Numbers were too small to evaluate the impact of gender or the underlying cause of possible gender-based difference in outcome.

Study 2 of 5: Rumbeiha WK, Oehme FW. Methylene blue can be used to treat methemoglobinemia in cats without inducing Heinz body hemolytic anemia. Veterinary and Human Toxicology 1992 Apr; 34(2): 120-2.

Design	**Goal:** To study the usefulness of methylene blue in the treatment of methemoglobinemia in cats.	**Comments:**
• Controlled study of induced toxicosis N = 40 (20 male and 20 female) *Sponsor(s):* American Academy of Clinical Toxicol-ogy	**Methods:** • Adult cats were given intravenous sodium nitrite, 1.5 mg/kg, to induce methemoglobinemia. Blood methemoglobin, CBC, total plasma protein, and fibrinogen were measured at intervals. **Dose and duration:** • Study duration was 15 days. • Group 1—Intravenous saline (negative control) Group 2—Intravenous methylene blue, 1.5 mg/kg Group 3—Intravenous methylene blue, 1.5 mg/kg, repeated in 4 hours Group 4—Intravenous sodium nitrite, 1.5 mg/kg Group 5—Intravenous sodium nitrite, 1.5 mg/kg, followed two hours later by intravenous methylene blue, 1.5 mg/kg Group 6—Intravenous sodium nitrite, 1.5 mg/kg, followed two hours and six hours later by intravenous methylene blue, 1.5 mg/kg **Results:** • Signs of toxicosis were induced in cats given sodium nitrite. Peak methemoglobin was 40 to 50%. However, it had dropped to 24 to 36% by the time of the first treatment and dropped to normal at 4 hours after sodium nitrite, regardless of whether treatment was given or not. • No signs of anemia were seen in any cat. The percentage of red blood cells with Heinz bodies in cats before treatment was 0 to 2%. This rose to 5 to 8% within the first eight days after a single dose of methylene blue to healthy cats and to 10 to 21% after 2 doses. In cats given sodium nitrite, followed by one dose of methylene blue, the peak Heinz bodies measured was 9% on the ninth day; when followed by two doses of methylene blue, it rose to 50%. **Conclusions:** • Methylene blue at a dose of 1.5 mg/kg can be used to reverse methemoglobinemia.	• This study was intended to investigate whether 1 or 2 doses of methylene blue could be given without significant toxicosis.

Study 3 of 5: Wilkie DA, Kirby R. Methemoglobinemia associated with dermal application of benzocaine cream in a cat. Journal of the American Veterinary Medical Association 1988 Jan 1; 192(1): 84-6.

Design • Case report N = 1	**Methods:** • Nine-month-old domestic shorthair presented for acute collapse. The owner had treated dermatitis with benzocaine skin cream. Mucous membranes were cyanotic. Heart and respiratory rates were increased. The blood sample taken was dark brown. Presumptive diagnosis was methemoglobinemia. **Dose:** • Intravenous methylene blue, 1.5 mg/kg • Additional treatment included dexamethasone sodium phosphate, intravenous fluids, and oxygen therapy. **Results:** • The cat responded within 5 to 10 minutes of methylene blue administration. Although it remained mildly cyanotic for 12 hours, it was released from the hospital within 24 hours. **Conclusions:** • Based on the initial assessment of increasing respiratory rate and worsening condition, followed by rapid response to methylene blue, the authors conclude this cat responded to methylene blue, rather than natural enzyme conversion of methemoglobin to hemoglobin.	

Study 4 of 5: Harvey JW, Keitt AL. Studies of the efficacy and potential hazards of methylene blue therapy in aniline-induced methaemoglobinemia. British Journal of Haematology 1983; 54: 29-41.

Design • Human case report and feline study of induced toxicosis with negative control N = 30 *Sponsor(s):* National Institutes of Health	**Goal:** To report on a human case of aniline poisoning and investigate the safety and effectiveness of methylene blue at a lose dose in cats with aniline-induced methemoglobinemia. **Methods and dose:** • Case report—Administration of methylene blue to a 22-year-old man with methemoglobinemia from ingestion of aniline oil did not relieve cyanosis; hemolysis followed. • Cats—Each cat was given a treatment, as outlined below. Then blood samples were taken intermittently for 9 days for hematocrit, methemoglobin percentage, erythrocyte GSH, Heinz body count, and hemolysate turbidity. Group 1—Given intravenous aniline at a dose of 18 mg/kg Group 2—Given aniline, followed 2 and 3 hours later by methylene blue at a dose of 2 mg/kg. Group 3—Given only methylene blue at the 2 and 3 hour times Group 4—Given aniline, followed 2 hours later by methylene blue Group 5—Given no medications **Results (feline study):** • Baseline methemoglobin for all cats was 0.54 ± 0.24% of total hemoglobin. Groups given aniline had >50% methemoglobin within 2 hours. • In group 2, methylene blue reduced methemoglobin to 10.7 ± 5.2% at 3 hours while the aniline-only group was 62.0 ± 6.9% at 3 hours. There was no statistical difference in methemoglobin response, Heinz body production, or packed cell volume (PCV) decrease between cats given one or two doses of methylene blue. • Within 3 days, PCV was significantly reduced in cats given methylene blue or aniline plus methylene blue, but not in cats given just aniline. • Cats given either aniline or methylene blue had a significant decrease in reduced glutathione within a day. **Conclusions:** • Cats appear to have a poor ability to remove erythrocytes with Heinz bodies from circulation; anemias were relatively mild compared to human beings with similar Heinz bodies present. • The results suggest that doses of methylene blue that are higher than 2 mg/kg may have no benefit and may increase adverse effects. • If methylene blue is used in the treatment of methemoglobinemia, patients should be monitored for anemia several days later.	

Study 5 of 5: Schechter RD, Schalm OW, Kaneko JJ. Heinz body hemolytic anemia associated with the use of urinary antiseptics containing methylene blue in the cat. Journal of the American Veterinary Medical Association 1973 Jan 1; 162(1): 37-44.

Design • Case series and toxicity study N = 6	**Methods:** • Case reports—Six cats were presented for severe drug-induced hemolytic anemia. They had been given a combination drug product for the treatment of urinary disease that included: methylene blue, atropine sulfate, hyoscyamine sulfate, gelsemiun, methenamine salol, and benzoic acid. They were presented after about 8 days (range, 2 to 15 days) of therapy. • Toxicity study—Consent was received to give 3 cats the medication while testing for Heinz body anemia. **Dose:** • Case reports—When the medication was given as directed, the cats received a dose of oral methylene blue that was about 3.5 mg/kg a day. • Toxicity study— A) One cat received about 3.5 mg/kg a day orally for 14 days. One received 3.5 mg/kg for two days, then 2.3 mg/kg for two days. The third cat was treated for 11 days, with doses varying daily. B) Two cats were subsequently given a medication for up to 50 days with all the same ingredients as that given to the case report cats, except that it did not contain methylene blue. C) Two cats were given methylene blue, 5.4 mg/kg every six to eight hours for nearly two days. **Results:** • Case reports—Each cat was presented for severe anemia (PCV <20%), with the exception of the cat that received only 2 days of treatment. Heinz bodies were identified in blood samples from each. Each responded to ending treatment with the combination product and beginning supportive treatment. • Toxicity study—Each cat developed Heinz body anemia and most gradually recovered once treatment ended. None of the cats receiving the medication without methylene blue developed Heinz bodies. **Conclusions:** • The severity of anemia induced by methylene blue appears to be dose-dependent.	

Methylene blue in the treatment of methemoglobinemia in dogs.

Revision date: June 28, 2008

Back to the indication.

The following tables are not intended to be in-depth reviews of each article. Instead, they are brief overviews/reviews of the studies, assuming reviewers are already familiar with the cited references. If you would like additional information about these studies, please contact veterinary@usp.org.

EFFICACY

Study 1 of 5: Harvey JW, King RR, Berry CR, et al. Methaemoglobin reductase deficiency in dogs. Comparative Hematology International 1991; 1: 55-9.

Design • Case reports N = 3	**Goal:** To describe methylene blue treatment of methemoglobinemia in dogs with naturally occurring methemoglobin reductase deficiency **Methods:** • Cases included a 2-year-old spayed female Toy Alaskan Eskimo dog, a 4-year-old spayed cocker/poodle mix dog, and a 3-year-old male miniature poodle. Each dog was presented for cyanosis induced by exercise, but packed cell volume and arterial partial pressure of oxygen ($p0_2$) were normal. Blood appeared brownish when exposed to air. • In each case, methemoglobinemia was confirmed by spectrophotometer and enzyme deficiency by ferricyanide reduction with NADH. Methemoglobin, as a percentage of hemoglobin, ranged from 19 to 36% in these dogs. • The three dogs were first given methylene blue. Two dogs subsequently received riboflavin, once their methemoglobin had climbed back to the baseline percentage. **Dose:** • Intravenous methylene blue, a single dose of 1 mg per kg of body weight (mg/kg), administered as a 1% solution • Oral riboflavin, 50 mg total dose every eight hours for one to two months. One dog was then given 75 mg every eight hours for 3 additional months. **Results:** • In all dogs, methemoglobin quickly dropped to 5% or less in the first hour after methylene blue administration and gradually increased over the next few days. No anemia or significant Heinz body formation, or other adverse effect, was noted in these three cases. • Riboflavin administration had no effect on methemoglobinemia. No adverse effects of treatment were noted. **Conclusions:** • Based on the rate of methemoglobin increase after methylene blue treatment in dogs, the typical rate of methemoglobin production, normally controlled by methemoglobin reductase, may be 2.5 to 3.2% a day. • Oral riboflavin was ineffective in dogs when administered at 20 to 80 times the dosage used to treat this deficiency in human beings, possibly because of poor oral absorption.	

SAFETY

Study 2 of 5: Osuna DJ, Armstrong PJ, Duncan DE. Acute renal failure after methylene blue infusion in a dog. J Am Anim Hosp Assoc 1990 Jul/Aug; 26: 410-2.

Design • Case report N = 1	**Goal:** To describe a dog that died of acute renal failure as a result of methylene blue infusion during surgery. **Methods:** • A 10-year-old spayed female miniature dachshund presented to North Carolina State University College of Veterinary medicine for workup. Primary hyperparathyroidism, pituitary-dependent hyperadrenocorticism, and allergic rhinitis were diagnosed. No evidence of anemia was found. Surgery for parathyroid tumor removal was performed. **Dose:** Intravenous methylene blue, 3 mg/kg, administered preoperatively in 250 mL of 0.9% saline solution, as an intraoperative stain **Results:** • Methylene blue did not preferentially stain the thyroid adenoma. • Within 24 hours, the dog developed intravascular hemolysis causing the packed cell volume (PCV) to drop from 54 to 39%. Jaundice, azotemia, and hyperamylasemia appeared 24 hours later but blood calcium and phosphorus were normal. Seventy-two hours after surgery, the PCV had declined to 25% and azotemia had worsened. Peritoneal dialysis was begun, but the dog died of respiratory arrest the next day. Necropsy and histology showed hemoglobinuric nephrosis secondary to intravascular hemolysis. **Conclusions:** • Chronic hypercalcemia may have predisposed to acute renal failure from hemoglobin exposure. • Methylene blue should be used with caution in geriatric dogs or dogs with renal disease.	

Study 3 of 5: Fingeroth JM, Smeak DD. Intravenous methylene blue infusion for intraoperative identification of parathyroid gland tumors in dogs. Part III. Clinical trials and results in three dogs. J Am Anim Hosp Assoc 1988 Nov/Dec; 24: 673-8.

Design • Prospective clinical trial N = 3	**Goal:** To describe clinical use of methylene blue infused at a dose of 3 mg/kg during surgery for primary hyperparathyroidism **Methods:** • Dogs referred for cervical exploratory surgery at Ohio State University Teaching Hospital. After the initial surgical exploration of the neck and elevation of the thyroid glands, methylene blue was infused to aid in identifying parathyroid tumors, involved regional lymph nodes, and ectopic parathyroid gland tissue. Hemograms and biochemistry were evaluated before surgery and intermittently for 8 days after surgery. No evidence of anemia was found before surgery. **Dose:** Intravenous methylene blue, 3 mg/kg, administered as an infusion over 30 to 40 minutes for intraoperative staining **Results:** • Pseudocyanosis was seen in two dogs during surgery. • PCV in all dogs declined within 12 hours of surgery and, for two dogs, a second drop to about 23% (from graph) occurred 2 to 4 days after surgery in conjunction with the appearance of Heinz bodies and red cell blistering. Blood transfusions were not given. • One anemic dog died five days after surgery; death was attributed to acute renal failure secondary to chronic hypercalcemia. **Conclusions:** • The authors recommend use of methylene blue, if needed to identify parathyroid neoplasia hidden by fat or the thyroid parenchyma, or to identify ectopic tissue. • Although one dog died, the authors felt anemia was not a complicating cause of death.	

Study 4 of 5: Fingeroth JM, Smeak DD. Intravenous methylene blue infusion for intraoperative identification of pancreatic islet-cell tumors in dogs. Part II. Clinical trials and results in four dogs. J Am Anim Hosp Assoc 1988 Mar/Apr; 24: 175-82.

Design • Prospective clinical trial N = 4	**Goal:** To describe clinical use of methylene blue infused at a dose of 3 mg/kg during surgery for insulinoma **Methods:** • Dogs referred for abdominal exploratory surgery at Ohio State University Teaching Hospital. After the abdomen was opened and initially explored, methylene blue was infused to aid in the identification of pancreatic nodules for wedge biopsy. Hemograms and biochemistry were evaluated before surgery and intermittently for 8 days after surgery. No evidence of anemia was found before surgery. **Dose:** Intravenous methylene blue, 3 mg/kg, administered as an infusion in physiologic saline over 30 to 40 minutes, for intraoperative staining **Results:** • Pseudocyanosis was seen in one dog during surgery. All dogs had green-tinged urine for 24 to 48 hours after surgery. • Two to three days after surgery, PCV declined to an average of less than half of preoperative values in all dogs. In two dogs, Heinz bodies and red cell blistering appeared during the PCV decline. In three dogs, PCV dropped below 30%. Blood transfusions were not given. • Three dogs developed acute pancreatitis. One dog developed a pancreatic abscess and died a week after surgery. **Conclusions:** • In these cases, methylene blue at a dose of 3 mg/kg was useful in identifying pancreatic islet cell tumors. • The dogs in this study appeared more sensitive to methylene blue toxicosis than healthy dogs, perhaps because they also experienced major organ surgery and some surgical blood loss. Anemia was a complicating factor in the death of one dog.	

Study 5 of 5: Fingeroth JM, Smeak DD, Jacobs RM. Intravenous methylene blue infusion for intraoperative identification of parathyroid gland and pancreatic islet-cell tumors in dogs. Part I. Experimental determination of dose-related staining efficacy and toxicity. J Am Anim Hosp Assoc 1988 Mar/Apr; 24: 165-73.

Design • Case report and random-ized, controlled dosing study N = 24	**Goal:** To assess the tissue staining characteristics of methylene blue during surgery in dogs and to evaluate the clinical effects **Methods:** • Case reports—Surgery was performed in one dog with parathyroid adenocarcinoma and one with suspected insulinoma, utilizing methylene blue as intraoperative stain. • Dosing study—Surgical midline exposure in healthy dogs of thyroid gland, parathyroid glands, and pancreas to score the staining achieved. Also separately evaluated time to maximum staining. Postoperative hematology and chemistry were evaluated intermittently for 14 days. **Dose:** Case reports—Intravenous methylene blue, 5 mg/kg Dosing study— • Group 1: No stain administered • Group 2: Intravenous methylene blue, 1 mg/kg, administered as an infusion in 250 mL of isotonic saline solution • Group 3: Intravenous methylene blue, 3 mg/kg, administered as in Group 2 • Group 4: Intravenous methylene blue, 5 mg/kg, administered as in Group 2 **Results:** Case reports—Both dogs had a decrease in PCV; Heinz bodies were observed. The PCV dropped from 43% to 18% in one dog and a blood transfusion was given. Dosing study— • Packed cell volume decreased 12 hours after surgery in all 4 groups, but there was a significant difference between treatment groups and the control group. In groups 2, 3, and 4, a larger drop in PCV occurred 3 to 4 days after surgery. In group 4, the most affected, the mean drop in PCV was to about 32% (from graph). No dog received a transfusion. • Mean reticulocyte counts were 76.7, 188.2, 173.1, and 368.1 (x 10^3), for groups 1 to 4, respectively. Frequent Heinz bodies were reported in 9 dogs but were not related to dose. Blistered red cells were reported to be infrequent in Group 2 and 3 dogs and frequent in Group 4 dogs. **Conclusions:** • The results suggest that 3 mg/kg would be an effective stain for this purpose and should be safe for clinical use.	

Methylene blue in the treatment of methemoglobinemia in horses.

Revision date: February 29, 2008

Back to the indication.

The following tables are not intended to be in-depth reviews of each article. Instead, they are brief overviews/reviews of the studies, assuming reviewers are already familiar with the cited references. If you would like additional information about these studies, please contact veterinary@usp.org.

Study 1 of 1: Medeiros LO, Nurmberger R, Medeiros LF. The special behavior of equine erythrocytes connected with the methemoglobin regulation. Comparative Biochemistry and Physiology 1984; 78B(4): 869-71.

Design • *In vitro* study using blood samples	**Goal:** To study the capacity of equine erythrocytes to prevent the formation of methemoglobinemia **Methods:** • Total (85 to 99%) and partial (25 to 40%) oxidation was induced by sodium nitrite in blood samples from 6 healthy Thoroughbred horses. The samples were incubated in a medium that contained glucose, methylene blue plus glucose, or lactate. • Methemoglobin concentration was measured after 2, 4, 6, and 24 hours. Enzyme activities were measured for glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, reduced glutathione, reduced nicotinamide adenine dinucleotide methemoglobin reductase, and reduced nicotinomide adenine dinucleotide phosphate methemoglobin reductase. **Results:** • Equine erythrocytes required a longer incubation time with glucose or methylene blue to reduce methemoglobin than human erythrocytes required. **Conclusions:** • This study contradicts previous studies of equine erythrocytes that reported poor ability to reduce methemoglobin. Equine erythrocytes efficiently reduced methemoglobin under conditions of partial oxidation. • Methemoglobin reduction was not enhanced by the presence of methylene blue.	