Molybdenum (Mo), an essential trace element required for nitrogen fixation and the reduction of nitrate to nitrite in plants and bacteria, is widely distributed in nature.\textsuperscript{214} Geochemical surveys in England found that Mo content in soil and sediment corresponds closely to underlying black shales.\textsuperscript{215} Other sources of Mo in the environment include industrial contamination by metal alloy manufacturing, copper mining, and coal mining.\textsuperscript{216-221} Molybdenum occurs predominately as the molybdate (MoO\textsubscript{4}\textsuperscript{2-}) ion in natural water sources, and concentrations are typically very low (<2-3 µg/L), unless contaminated by an outside source, in which case they can reach 25 mg Mo/L.\textsuperscript{222,225} In forage, Mo concentrations vary and depend on the Mo concentration, moisture content, and the pH of the soil.\textsuperscript{222,224} Alkaline environments greatly increase the bioavailability of Mo to plants, and thus increase the likelihood of Mo toxicity in grazing animals.\textsuperscript{222,225} Surveys have identified extensive areas of forage containing potentially toxic concentrations of Mo (10-20 ppm) in at least five western states, including Wyoming.\textsuperscript{226}

**Essentiality**

Mo is an essential element for mammals due to its involvement in the enzymes aldehyde oxidase, sulfate oxidase, and xanthine oxidase.\textsuperscript{214,227} As a cofactor for these enzymes, it aids in catalyzing the oxidation or metabolism of sulfur-containing amino acids, purines, pyrimidines, and aldehydes.\textsuperscript{214} Experimentally, dietary Mo deficiency decreased feed consumption and caused a 25% reduction in live-weight gains in adult goats, and kids from deficient dams gained less compared to control animals.\textsuperscript{228,229} Reproductive effects of deficiency include decreased pregnancy rates and higher mortality in offspring.\textsuperscript{227-229} Dietary requirements are so low (about 100 ppb DM), however, that deficiency is very rare under natural conditions.\textsuperscript{227}

**Metabolism**

Once ingested, Mo is absorbed in the stomach and throughout the small intestine. In the small intestine, MoO\textsubscript{4} is actively transported across the mucosal epithelium via the same carrier-mediated transport mechanism that transports sulfate (SO\textsubscript{4}\textsuperscript{2-}).\textsuperscript{230} The administration of SO\textsubscript{4} to monogastric animals consuming a Mo-rich diet decreases blood Mo concentration and increases excretion, potentially alleviating toxicity.\textsuperscript{230-234} Interestingly, dietary S increases the toxicity of Mo in ruminants, presumably as a result of thiomolybdate formation in the rumen. Molybdenum is transported in the blood as MoO\textsubscript{4}, where it is distributed to tissues for integration into enzyme systems. Excretion is primarily via urine; however, feces and milk can also serve as important routes of removal.\textsuperscript{214,235-238} The rate of absorption differs amongst species, age groups, and sex. For example, after orally administering Mo to swine and cattle, it was found that Mo peaked in the blood of swine after two to four hours, while it took 96 hours to peak in cattle.\textsuperscript{236} Water soluble forms of Mo, such as ammonium molybdate ((NH\textsubscript{4})\textsubscript{6}Mo\textsubscript{7}O\textsubscript{24}), sodium molybdate (Na\textsubscript{2}MoO\textsubscript{4}), and Mo from forage, are more readily absorbed than their organic counterparts.\textsuperscript{214} The ruminal interaction between Mo, SO\textsubscript{4}, and Cu is responsible for the greater sensitivity of ruminants than monogastrics to Mo. In the rumen, sulfur compounds (mostly SO\textsubscript{4}) are reduced to sulfide by rumen microbes. Sulfide then combines with MoO\textsubscript{4} to form either tri- or tetrathiomolybdates (TM).\textsuperscript{214,239,240} Thus, in ruminants, relatively little MoO\textsubscript{4} or S reach the small intestine to be absorbed as such. In the rumen, TMs bind irreversibly to the solid phase of digesta and act as powerful chelators of Cu, retaining it in the gut. As a result, Cu absorption from the GI tract is decreased as much as 88%.\textsuperscript{241-245} Trithiomolybdates can also enter the circulation where they bind cooperatively with Cu to albumin, resulting in decreased availability of Cu for critical functions. Because this complex is insoluble in trichloroacetic acid (TCA), the term “TCA-soluble” Cu is often used as a synonym for biologically useful Cu.\textsuperscript{241,245,247-249} As a consequence of the binding to albumin, biliary excretion of Cu is enhanced, less Cu is incorporated into enzymes such as ceruloplasmin (Cp), and less Cu is stored in tissues. The decreased availability of Cu for enzyme synthesis impacts a number of physiologic processes, including immune function and bone and elastin formation.\textsuperscript{242,245,250-261} Trithiomolybdates bound to albumin are
relatively stable; however, once unbound, TMs are rapidly hydrolyzed to \( \text{MoO}_4^2- \) and \( \text{SO}_4^{2-} \).\(^{246,249,262}\)

Since the clinical effects of molybdenosis are in large part due to secondary Cu deficiency, it is useful to review Cu metabolism. Although Cu is an essential trace element for all mammals and deficiency is frequently associated with a number of maladies, the Cu ion itself is quite toxic to cells. Thus, the metabolism of Cu in mammals involves a number of different carrier and storage proteins that bind Cu, permitting it to be absorbed, distributed, and eliminated without exposing cells to excessive amounts of the free ion. Copper is absorbed in the intestine by carrier proteins and stored in mucosal cells as a protein complex.\(^ {265}\) In monogastric species such as rats, horses, and swine, most uptake occurs in the small intestine. In ruminants, there is some evidence significant absorption also occurs in the large intestine.\(^ {245}\) Copper is transported to the liver bound to albumin and transcrupein, where the proteins and their bound Cu atoms are taken up into hepatocytes.\(^ {265}\) Within the liver cells, Cu is distributed between various storage proteins, especially metallothionein (MT), microsomes, nuclei, lysosomes, and the cytosol.\(^ {245,265}\) Copper is exported from the liver to the rest of the body for incorporation into enzymes as a protein complex with Cp.

### Toxicity

Despite the fact Mo is intrinsically (i.e. without metabolism to TM) toxic\(^ {245,264,265}\), secondary Cu deficiency is the most common pathogenesis underlying molybdenosis. The form of Mo ingested and, more importantly, the Cu:Mo ratio are critical determinants of toxicity.\(^ {266-268}\) Cu:Mo ratios of 2:1 or less result in clinical signs, and effects are exacerbated by high dietary S.\(^ {267}\) Various authorities have recommended Cu:Mo ratios of 4:1 or greater as the minimum “safe” ratio.\(^ {214,267,269}\) Signs of acute Mo toxicity include gastrointestinal irritation, diarrhea, liver and kidney damage, and, ultimately, death.\(^ {253,267,270}\) Diarrhea appears to be a direct effect of Mo on the intestinal mucosal cells, rather than Cu deficiency.\(^ {245}\) In chronic poisoning, anorexia and weight loss are initial clinical signs, followed by diarrhea, anemia, depigmentation of the hair coat (achromotrichia), ataxia, and bone and joint deformities.\(^ {214,267,271,272}\) Integumentary lesions and bone and joint deformities are probably due to deficiencies of several Cu-dependent, critical enzymes, as well as possibly decreased P in bone.\(^ {267,273,274}\) Decreased reproductive function, including decreased libido and fertility, has also been associated with molybdenosis.\(^ {266,275-278}\) Acute to subacute toxicity has been demonstrated experimentally and occurs naturally in cattle, buffalo, and mule deer. The accidental addition of \( \text{Na}_2\text{MoO}_4 \) at the rate of 19,000 ppm (estimated Mo concentration 7,400 ppm) to cattle rations resulted in decreased feed intake, hind limb ataxia, profuse salivation and ocular discharge, diarrhea, liver and kidney damage, rough hair coat, and death.\(^ {270}\) Feeding 1.36 g Mo per head per day to five cows for an unspecified amount of time produced extreme scouring and loss of condition in three animals.\(^ {279}\) After consuming a ration containing 10.5 ppm Cu and 140 ppm Mo for three to four days, Holstein-Friesian lactating cows and steers developed hemorrhagic diarrhea and front limb lameness, and they died.\(^ {280}\) Contaminated grazing pastures with forage Mo concentrations between 16-24 ppm and 6-11 ppm Cu resulted in acute diarrhea, loss of condition, and posterior stiffness in cattle.\(^ {221}\) Feeding 2,000 ppm Mo as \( (\text{NH}_4)_2\text{MoO}_4 \) for three days resulted in diarrhea and feed refusal in cattle.\(^ {281}\) After grazing a pasture contaminated with used motor oil containing molybdenum bisulfide for two weeks, cattle exhibited diarrhea, anemia, decreased milk production, achromotrichia, and hind limb weakness.\(^ {282}\) Four male buffalo were given 5 mg \( \text{Na}_2\text{MoO}_4 \) (2.35 mg Mo)/kg BW/day for 180 days; by two weeks, clinical signs included diarrhea, decreased weight gains, incoordination, swelling of hind fetlocks, and irregular hoof wear.\(^ {283}\) Including 2,500 ppm Mo as \( \text{Na}_2\text{MoO}_4 \) (equivalent to approximately 62.5 mg Mo/kg BW) or more in the diets of mule deer for 33 days resulted in diarrhea and feed refusal; whereas, smaller doses were without apparent effect. The authors concluded that deer are relatively resistant to Mo, compared to cattle.\(^ {284}\)

Relatively few studies of molybdenosis have been conducted in monogastrics. Most of these involve relatively large doses of Mo and don’t seem to involve Mo-Cu-S interactions.\(^ {233,285-288}\) Rats given a diet containing 1,000 mg \( \text{Na}_2\text{MoO}_4 /\text{kg BW} \) for five weeks gained significantly less than controls, and 50% developed mandibular exostoses.\(^ {289}\) Feeding rats 1,200 ppm Mo as \( \text{Na}_2\text{MoO}_4 \) for six weeks resulted in decreased weight gains, and skin, tail rings, and femurs all required much less force to break compared to control tissues.\(^ {290}\) Six hundred ppm Mo fed to rats for three weeks caused a depression in nutrient utilization and decrease in gain.\(^ {296}\) Rats consuming 800 ppm Mo for five weeks showed a 36% depression in growth; adding 0.29% S, largely prevented this effect.\(^ {233}\) Male rats given 1,200 ppm Mo as \( \text{Na}_2\text{MoO}_4 \) for four to five weeks showed a 53% reduction in growth as...
well as reduced feed intake, followed by death. One thousand ppm dietary Na₄MoO₄ resulted in weight loss, diarrhea, hair changes, palpable mandibular nodules, and other bone and joint abnormalities in rats. Rats fed 20 ppm Mo and 5 ppm Cu showed significant reduction in gains and depigmentation of hair. Rabbits consuming 1,000 ppm Mo as Na₂MoO₄ in a diet with 16.4 ppm Cu developed anorexia, alopecia, slight dermatosis, and anemia, and they died after four weeks. Feeding rabbits 4,000 ppm Na₂MoO₄ (roughly 55 mg Mo/kg BW) for four weeks resulted in anemia, abnormal bone development, and degeneration of myocardium and skeletal muscles. Ponies fed up to 102 ppm Mo with normal (9 ppm) dietary Cu remained asymptomatic for 50 days. Similar results were reported in colts fed 50 ppm dietary Mo. Four horses fed 20 ppm dietary Mo as 79Mo did not exhibit any signs of molybdenosis or deranged Cu metabolism. The radioactive label in plasma was bound to the MoO₄ ion rather than TM, as seen in ruminants.

Chronic toxicity has been investigated both in field studies and experimentally in cattle, sheep, and ruminant wildlife. Forage containing 24-28 ppm Cu and 14-126 ppm Mo that had been contaminated by a metal alloy manufacturing plant resulted in emaciation and diarrhea in cattle beginning four weeks after introduction to the affected pasture. Cattle grazing pasture contaminated by aluminum alloy plants at a concentration of 77.5 ppm Mo exhibited severe scouring, loss of condition, decreased milk production, and achromotrichia. Cattle grazing pastures containing 1-20 ppm Mo exhibited diarrhea, listlessness, and abnormal hair color compared to animals grazing pastures containing < 1 ppm Mo with no clinical signs. Feeding two male calves, with average body weights of 460 pounds, 4.0 g Na₂MoO₄/day resulted in diarrhea, discolored hair, weight loss, anemia, and decreased libido. Cattle consuming a diet containing 4 ppm Cu and 5 ppm Mo exhibited decreased weight gains, decreased feed intake, and abnormal hair texture and color after 16-20 weeks. Pastures containing 25.6 ppm Mo produced severe diarrhea, emaciation, anemia, faded hair color, salt craving, and death in grazing cattle. Yearling steers given 1.5 mg Mo/kg BW for 150 days and grazed on pasture containing 0.32% S developed diarrhea, inflammation of the sheath, rough hair coat, and anemia.

Grazing forage containing 4-14 ppm Cu and 95-460 ppm Mo and drinking water with 14 μg Cu/L and 7,200 μg Mo/L for 11 weeks resulted in watery diarrhea, rough hair coats, and a stiff shuffling gait in 50% of cow-calf pairs. Forage contaminated with 2-220 ppm Mo caused diarrhea, roughening and discoloration of the hair coat, and weight loss in grazing cattle. Forage concentrations of 16.5-23.5 ppm Mo and 2-25 ppm Cu resulted in diarrhea, weight loss, and achromotrichia in cattle. Feeding 100 ppm Mo to heifers for 11 months resulted in anemia, scurving, achromotrichia, and weight loss. Five of 16 died two weeks after termination of the experiment. Heifers receiving diets containing 100 ppm Mo and 0.3% S as SO₄ became emaciated and diarrheic after one month at this treatment level. Heifers given 5-20 ppm Mo with 0.3% S or 50 ppm Mo without added S did not exhibit any signs of illness, but they did have decreased tissue Cu compared to controls. Weight loss and scouring were evident in cows ingesting 173 ppm Mo for two months. Diets containing 53 and 100 ppm Mo did not produce clinical signs but did interfere with Cu metabolism. Forage containing 6-36 ppm Mo resulted in emaciation in cattle with severe diarrhea, anemia, achromotrichia, and swollen genitals. Water containing 50 ppm Mo induced signs of secondary Cu deficiency in five-week-old calves. Moose in Sweden have been reported to exhibit signs of molybdenosis similar to cattle, including diarrhea, emaciation, achromotrichia, sudden heart failure, and osteoporosis; however, no levels of Mo in the forage or water sources were reported.

Sheep grazing pastures containing 5.5-33.5 ppm Mo and 6.0-8.7 ppm Cu for 76 weeks developed hemorrhaging around the femoral heads, tuber sacrales, and psoas muscles. Exostoses were frequent on humeri and femurs, and the periosteum appeared to have lifted from the bone surface. Feeding ewes 1 kg commercial grass cubes with 5 ppm Cu and supplemented with Na₂SO₄ and (NH₄)₆MoO₂₄ to provide 10 g SO₄ and 50 mg Mo, respectively, until one month prior to lambing resulted in incoordination of front and hind limbs and a marked ataxia in lambs within 60 days of birth. Sheep grazing pastures with 20 ppm Mo and 5-7 ppm Cu developed connective tissue lesions including lifting of the periosteum and hemorrhaging in periosteum and muscle insertions. Drenching (i.e. dosing by stomach tube) goats daily with (NH₄)₆MoO₂₄ to provide 50 ppm dietary Mo for 235 days caused general debility, depigmentation of hair coat, and weight loss.

Notwithstanding other work, several studies have failed to produce any adverse health effects after Mo ingestion. Grazing cattle on pastures containing 13 ppm Mo and
supplementing with 17 ppm Cu for six months resulted in no signs of adverse effects. Cattle grazing a re-claimed mine tailing site containing 21-44 ppm Mo and 13-19 ppm Cu for three consecutive summers exhibited no signs of Mo toxicity. No adverse effects were noticed in a cannulated steer consuming sun-cured hay containing 49.68 ppm Mo and 19.09 ppm Cu. In each of these studies, low S in both the diet and water was offered as being a possible explanation for the lack of clinical signs observed.

Summary

Horses, like most monogastric animals, are very resistant to the effects of Mo as compared to ruminants. Cattle are commonly cited as slightly more susceptible to molybdenosis than sheep, and limited quantitative data suggest mule deer are relatively resistant compared to cattle. Therefore, drinking water Mo concentrations that are safe for cattle are probably also safe for horses, other classes of livestock, and wild cervids. Although there is quite a bit of variability in the reports summarized above, and some (large) amount of dietary Mo may cause poisoning regardless of Cu status, the bottom line seems to be that total dietary Cu:Mo ratios of less than 2-4 can result in chronic toxicity and decreased production in cattle, especially if dietary S is higher than absolutely necessary.

As with many substances, the effects of forage and water Mo concentrations are additive, and, in some areas of the western United States, forage Mo concentrations are already toxic or very nearly toxic. Under these conditions, any additional Mo intake contributed by drinking water is potentially dangerous. In these areas, however, producers are likely already aware of the problem and feeding supplemental Cu. A more normal situation would be cattle grazing “typical” Wyoming forage containing 7 ppm Cu (or supplemented to that level) and negligible Mo (<1 ppm). Under these conditions the critical safe 4:1 ratio would be exceeded whenever drinking water contains 375 μg Mo/L.

We recommend that, in the absence of other data, drinking water for livestock and wildlife contain less than 0.3 mg/L. If dietary Mo is higher, which is not unusual in this region, water Mo concentrations should be adjusted downward accordingly.