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Risk Assessment of Copper and Molybdenum and Other Minerals in Feed Ingredients and Finished Feeds

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Abstract

This is a risk assessment for six mineral contaminants and four essential minerals. A total of 1,466 samples were collected from 18 product categories during 2010 to 2018. The 18 product categories were divided into 11 finished feed (689 samples) categories and seven feed ingredient categories (777 samples). Mineral and vitamin/minerals mixes (565 samples) were the predominant products in the seven feed ingredient categories, and beef cattle feed (351 samples) was the predominant product in the 11 finished feed categories. Samples from the 18 product categories were analyzed for up to 10 minerals: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), molybdenum (Mo), mercury (Hg), nickel (Ni), selenium (Se), and thallium (Tl). Cr, Cu, Mo, and Se are essential nutrients, and the other six are contaminants. The ratios of mean concentrations of the 6 inorganic contaminants across the 11 finished feeds were low compared to the seven feed ingedients categories. This implies that the risk of toxicity from the potential carryover of the six contaminants from feed ingredients to finished feeds is low. The mean Cu concentrations were high and the mean Mo concentrations were low in the four finished feed categories for ruminants (cattle, sheep, and goats). The ideal copper:molybdenum (Cu:Mo) ratio is >6:1 and <10:1 in the four finished feed categories for ruminants. The calculated Cu:Mo ratio of 26:1 shows a nutritional imbalance between Cu and Mo that may cause Cu toxicosis with long-term exposure.

Keywords: animal feed, hazard, risk assessment, copper, molybdenum, contaminants, minerals, essential minerals, feed ingredients, finished feeds

1. Introduction

Feed ingredients such as mineral mixes (supplements) may contain inorganic contaminants that are naturally occurring minerals but are toxic to animals. Mineral contaminants in feed ingredients such as mineral mixes may be introduced during the manufacture of food products for animals [2]. Food animals contribute around 12.9 percent of global calories and 27.9 percent of protein through meat, milk, eggs, and offal [17].

Wastage of animal food can be reduced by managing the risk of feed contaminants, including collection of data on the presence of microbial contaminants or residues of mycotoxins, heavy metals, antibiotics, and pesticides, resulting in increased transparency for risk management at the national level [17]. Potential carryover of contaminants from feed to animal food products is an important aspect of the animal production chain [33]. Thus, it is important to monitor inorganic contaminants and essential minerals to ensure both animal and human health. Information on such carryover is sparse in the scientific literature for inorganic contaminants in feed ingredients and finished feeds. In 1977, As, Cd, Pb, Hg, and vanadium (V) were evaluated to assess the potential injury to animals ingesting these toxic minerals from ores and industrial processes that provided 14 essential minerals [3]. Concentration of key elements, such as Pb, in meat and bone meal was measured in a North American study [11]. Cu and zinc (Zn) were above the recommended levels in Wisconsin dairy farms, and Cd was generally present in animal feeds [16]. In England and Wales, Nicholson et al. [28] evaluated As, Cd, Cr, Pb, Hg, and thallium (Tl) in feeds and animal manure. Mean concentrations of some of these contaminants were 0.19 mg/kg of feed for dairy cattle, 1.79 mg Cd/kg for minerals, 42 mg Cr/kg for minerals, and <1 mg Pb/kg for corn silage in the United Kingdom. Cd, Pb, and other inorganic contaminants may occur during processing of feeds and from environmental pollution [34, 35]. Some mineral premixes that are co-products of industrial metal production can be contaminated with heavy metals [13]. Organoarsenicals in poultry litter used as a feed ingredient for ruminant diets may

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be a source of As contamination in finished feeds [18]. However, organoarsenicals for use in poultry and swine as coccidiostats and growth promoters were voluntarily withdrawn from the United States (U.S.) market in 2011 and banned in Canada, Malaysia, Australia, and the European Union [18].

Hazardous elements consisting of Cu, Zn, iron (Fe), manganese (Mn), cobalt (Co), Ni, Cr, Pb, Cd, and Hg were analyzed in 15 samples of finished feeds, and compared to the maximum limits (ML) under the regulations of the Slovak Republic and the European Commission (EC) [30]. The concentration of Cu, Mn, Zn, and Fe in the 15 samples were mostly below the legislative limits set by the EC. Three of the 15 samples had concentrations of Co, Ni, Pb, Cr, or Cd above the legislative limit set by the EC for high producing dairy cattle. In Spain, 435 mineral premix samples were tested for Cd, Pb, As, and Hg to compare the concentration of these heavy metals with the regulatory limits set by the European Commission (EC) Directive 2002/32/EC [13]. Overall, 21 percent (92 of the 435 samples) showed high concentrations of heavy metals above the European regulatory limits. In the Netherlands, approximately 3,000 samples from a variety of feed ingredients and finished feeds were analyzed for As, Cd, Pb, and Hg to guide national monitoring [1]. The concentration of most of the samples was below the ML set by the EC and some slightly exceeded the ML. The Dutch study showed that monitoring should focus on eight feed ingredients: minerals, fish meal, seaweed, algae, Cu and Zn mineral premixes, and binders/anticaking agents.

In the U.S., copper and chromium salts, such as copper chloride, chromium propionate, and chromium tripicolinate, are generally recognized as safe (GRAS) food additives in animal diets [2]. Selenium is an approved food additive per U.S. Food and Drug Administration (FDA) regulation 21 CFR § 573.920 that allows the use of sodium selenite, sodium selenate, or selenium yeast as sources of selenium supplementation for chickens, swine, turkeys, sheep, cattle and ducks at the maximum level of 0.3 ppm (mg/kg) of complete feeds (finished feeds). Molybdenum is neither GRAS nor an approved food additive in the U.S.

The European Directive 2002/32/EC sets regulatory ML for As, Cd, fluorine (F), Pb, and Hg in feed ingredients and finished feeds for food-animals and pet food, but not other inorganic contaminants, such as Ni and Cr [8]. The U.S. does not have regulatory limits for inorganic contaminants in feed ingredients and finished feeds. Instead, the FDA relies on the maximum tolerable level (MTL) for 38 minerals in finished feeds based on the scientific literature [26, 8]. In general, the MTL pertains to animal health but not human health. Two of the reports provided by European Directive 2002/32/EC relate the concentration of a maximum of five inorganic contaminants in feed ingredients and finished feeds to their potential toxicity using the ML [30, 1]. None of the reports in the scientific literature compare the concentration of inorganic contaminants in feed ingredients and finished feeds to any of the 38 MTLs. The intent of this risk assessment is to conduct a qualitative risk assessment [10] of six inorganic contaminants and four essential minerals in seven feed ingredient categories and 11 finished feed categories.

2. Material and Methods

2.1. Reagents and materials

Reagent-grade chemicals were used unless otherwise specified, and deionized water was used to prepare the reagents and materials [8]. Nitric acid and hydrochloride acid solutions were all of trace metal grade and obtained from VWR (Radnor, PA). The matrices for 689 samples from seven feed ingredient categories were minerals, vitamin/minerals, fish meal, miscellaneous ingredients, cottonseed products, non-protein nitrogen, and rice products. The matrices for 777 samples from 11 finished feed categories were beef cattle feed, fish feed, swine feed, sheep and goat feed, horse feed, dairy cattle feed, liquid feed (cattle), wildlife feed, dog and cat food, and miscellaneous foods/feeds.

2.2. Sample collection

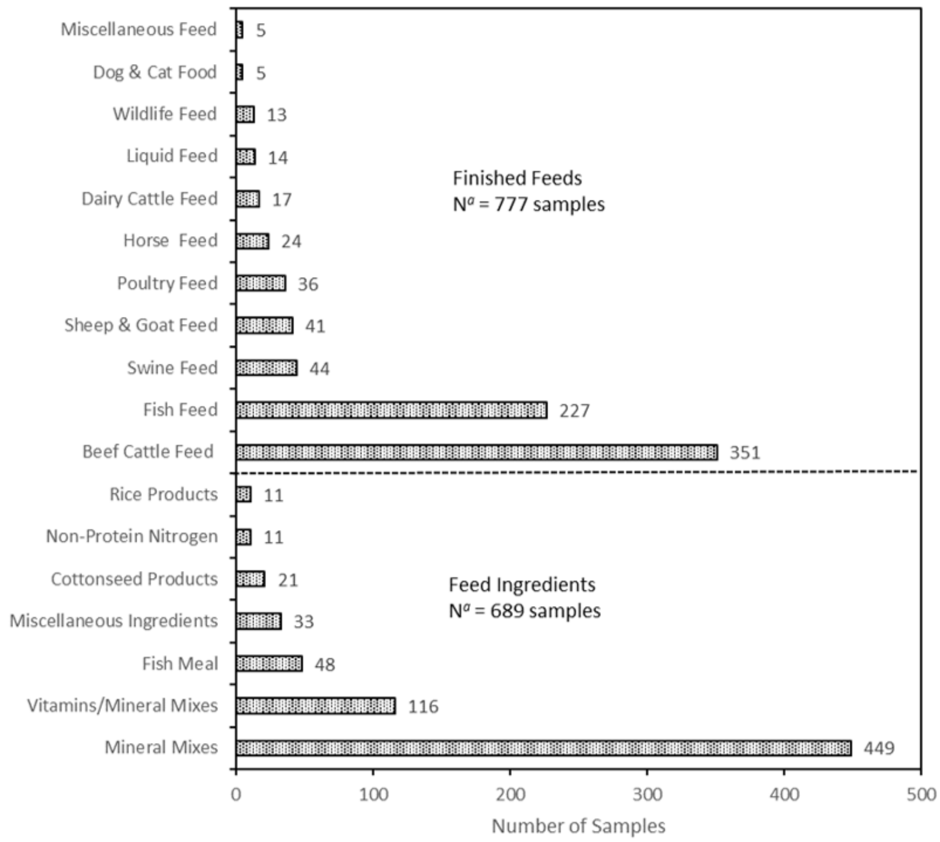
All regulatory samples for the 17 product categories were collected in the state of Texas from various manufacturers and distributors. Samples from the finished feed category for beef cattle were collected using the Office of the Texas State Chemist sample collection, preparation, and transport standard operating procedures [8]. Other samples were collected based on the procedures outlined in the AAFCO inspector's manual [2]. A minimum of 500 g of each sample were collected using a sterile scoop or trier, placed in a plastic lined paper bag, and shipped to the laboratory for analysis from 2010 through 2018.

2.3. Sample preparation and analysis

Samples were split using a commercial riffler (Carpco SS-16-25) [8]. The riffled unground samples were ground through a commercial grinder (Retsch Ultra Centrifugal Mill ZM 200, Haan, Germany) using a 0.75-mm diameter screen. The ground samples were then used for laboratory analysis. A 0.5-g portion of each dry feed sample was predigested in 3:1 HNO_3:HCl overnight before microwave digestion (MARS Xpress, CEM Corp, Matthews, NC). The digestion was performed at 200 °C, followed by cooling and dilution with deionized water. The diluted samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS Nexion 300 X, Perkin Elmer, Waltham, MA).

2.4. Statistical data analysis

The mineral concentrations below the limit of quantification (LOQ) for the analytical method were set to zero for the purpose of simplifying the statistical calculations of the mean and standard deviation (SD). The LOQ was defined as approximately three times the limit of detection (LOD) for each element [8]. The present study includes data from a previous surveillance study conducted from 2011 to 2016 [8].



[a]N = Total number of samples

Figure 1: Number of samples collected from the 18 product categories that were divided into seven feed ingredient categories and 11 finished feed categories.

3. Results

3.1. Feed ingredients and finished feeds

A total of 1,466 samples were collected from 2011 through 2018 from 18 product categories (Figure 1). The 18 product categories were divided into the seven feed ingredient categories, with a total of 689 samples, and the 11 finished feed categories with a total of 777 samples. Mineral and vitamin/mineral mixes with a total of 565 samples were the two predominant feed ingredient categories, and beef cattle feed with 351 samples was the predominant finished feed category. The miscellaneous feed category contained only five rabbit feeds, and the wildlife feed category contained mostly deer feed. The miscellaneous ingredient category was represented by a total of 33 samples of wheat hay, crab meal, fish oil, and clay binders. Cd and Pb were the only analytes for 227 samples from the fish feed category, and five to 10 elements were analyzed for the remaining 550 samples from the other 10 finished feed categories (Table 1 and Figure 1). If the number of samples was ≤5, the samples from the original seven feed ingredient categories and the 11 finished feed categories were incorporated into miscellaneous ingredients and miscellaneous feed categories, respectively.

3.2. Contaminants

The toxicity of the six contaminants was extensively discussed by Dai et al. [8] and will not be reiterated here. For the seven feed ingredient categories, mineral and vitamin/mineral mixes were the major sources of the six contaminants (As, Cd, Pb, Hg, Ni, and Tl) that are potentially carried over into the 11 finished feed categories (Table 2 and Figure 1). The ratios of the concentrations of five of the six contaminants across the 11 finished feed categories, compared to the mean concentrations across the seven feed ingredient categories, were relatively low, with a range of 0.02 to 3.7 percent, except for Ni (12 percent). To further explore the impact of Ni on animal health, a risk estimate was calculated for the six contaminants by multiplying the hazard score by the exposure score (Table 3). The hazard score is an estimate of toxicity based on an AAFCO ranking of mineral toxicity [2]. The exposure score is derived from the concentration of the contaminants in nine finished feed categories as an arbitrary estimate of long-term exposure. The risk estimates for the six contaminants were low, ranging from 2 to 4 out of a maximum of 12 (high risk) (Table 2). The risk estimate for Ni was 4 (low risk), which reflects its low toxicity and exposure score of 2 compared to the other five contaminants that are generally more toxic but have a low exposure score of 1 (Table 2).

Mineral	Combined Categories	N [a]	Mean (ppm) [b]	SD [c]	Maximum (ppm)
Contaminants					
Arsenic	Feed Ingredients	690	5.57	35.67	891.00
	Finished Feeds	549	0.04	0.28	3.50
Cadmium	Feed Ingredients	685	1.02	2.26	18.00
	Finished Feeds	775	0.03	0.15	3.00
Lead	Feed Ingredients	687	6.85	24.09	349.00
	Finished Feeds	775	0.25	1.32	35.00
Mercury	Feed Ingredients	496	0.02	0.09	0.76
	Finished Feeds	535	0.00	0.01	0.11
Nickel	Feed Ingredients	417	29.47	75.42	810.00
	Finished Feeds	533	3.41	8.44	72.00
Thallium	Feed Ingredients	525	0.12	0.93	14.10
	Finished Feeds	545	0.00	0.00	0.01
Essential Minerals					
Chromium	Feed Ingredients	419	25.59	60.44	539.00
	Finished Feeds	539	4.99	4.65	28.00
Copper	Feed Ingredients	525	24,871.21	73,460.28	277,000.00
	Finished Feeds	545	31.22	48.00	371.00
Molybdenum	Feed Ingredients	418	4.84	28.88	579.00
	Finished Feeds	533	1.22	0.81	4.20
Selenium	Feed Ingredients	522	92.71	503.69	10,300.00
	Finished Feeds	551	0.21	1.81	22.00

[a]N = Number of samples
[b]ppm = mg/kg
[c]SD = Standard deviation

Table 1: Mean concentrations of the six contaminants and the four essential minerals across the seven feed ingredient categories and 11 finished feed categories.

3.3. Essential minerals

Cr, Cu, Mo, and Se are essential minerals [26] that are added to animal diets, mainly through the two vitamin/mineral mixes categories, with some contribution from the remaining five non-mineral feed ingredient categories (Figure 1). As approved food additives, increased concentrations of Cr, Cu, and Se are expected in finished feeds. The ratios of mean concentration of Cr, Cu, Mo, and Se across 11 finished feed categories, compared to the mean concentrations across the seven feed ingredient categories, ranged from 0.22 to 25 percent (Table 2 and Figure 1). The high ratios of mean concentrations for Cr (20 percent) and Mo (25 percent) across the 11 finished feed categories, compared across the seven feed ingredient categories, imply toxic amounts. However, the risk estimates for Cr and Mo were low at 1 and 3, respectively, but the Cu risk estimate of 9 was high (Table 2). In the nine finished feed categories (Table 2), the mean concentrations of Se were below the MTL for eight out of nine finished feed categories. Beef cattle feed had seven out of 352 (3 percent) samples with Se concentrations greater than the MTL of 5 ppm, with a maximum of 22 ppm. Overall, the 11 finished feed categories did not contain excess amounts of Cr, Mo, and Se but may contain excess to toxic amounts of Cu.

3.4. Copper concentrations in finished feeds

The National Research Council (NRC) has not set a copper MTL for wildlife, dogs, and cat feeds. The NRC recommends using data for sheep and goats until controlled studies can estimate requirements for cervid (deer) species [27]. However, deer are more resistant than other ruminants (sheep), but less resistant than non-ruminants (monogastrics) to Cu poisoning [4, 7, 15]. Exposures of 24 to 40 ppm Cu in the cervid diet are well tolerated for long periods, and up to 220 ppm of dietary Cu in the short term. The cattle MTL of 40 ppm for Cu and 5 ppm for Mo are used for wildlife feed (deer) as reasonable estimates based on the scientific literature (Table 3). Likewise, a Cu MTL of 250 ppm and a Mo MTL of 150 ppm are reasonable estimates for dog and cat food as monogastric species.

For all species, the ratios of the mean concentration of Cu in nine finished feed categories compared to the MTL ranged from 8 to 153 percent (Table 3). For monogastric species, the ratios of the mean concentration of Cu in five finished feed categories compared to the MTL ranged from 8 to 27 percent (Table 3). For the ruminant species (cattle, sheep, goats, and deer), the ratios of the mean Cu concentration in four finished feeds, compared to the MTL, ranged from 64 to 153 percent (Table 3). The low ratios of mean Cu concentrations compared to the MTL

Mineral	Finished Feeds/ Feed Ingredients Ratio [a]	Mean Concentration in Finished Feed (ppm)	Hazard Score [b]	Exposure Score [c]	Risk Estimate [d]
Contaminants					
Arsenic	0.63%	0.04	2	1	2
Cadmium	3.40%	0.03	4	1	4
Lead	3.68%	0.25	3	1	3
Mercury	2.19%	0.00	4	1	4
Nickel	11.57%	3.41	2	2	4
Thallium [e]	0.02%	0.00	4	1	4
Essential Minerals					
Chromium	19.51%	4.99	1	1	1
Copper	0.13%	31.22	3	3	9
Molybdenum	25.24%	1.22	3	1	3
Selenium	0.22%	0.21	4	1	4

[a]Finished feeds/feed ingredient ratio = mean concentration of each mineral across 11 finished feeds divided by mean concentration of the respective mineral across seven feed ingredients x 100 percent

[b]Hazard score: Slightly Toxic = 1, Moderately Toxic = 2, Toxic = 3, Highly Toxic = 4 [2]

[c]Exposure scores are estimates derived from the mean concentrations in finished feed: Low ($<$1 ppm) = 1, Medium ($>$1 $<$10 ppm) = 2, High ($>$10 ppm) = 3

[d]Exposure estimate = hazard score x exposure score

[e]AAFCO did not designate a hazard score for thallium. The hazard score for thallium is based on its high toxicity.

Table 2: Risk estimate of the ten minerals as hazards in finished feed.

of 250 ppm for the monogastric species (horse, swine, poultry, and miscellaneous feed categories) reflects their resistance to Cu toxicosis (Table 3). The ratios of the mean Cu concentration compared to the respective MTL that were greater than 60 percent suggest an excess of Cu in ruminant diets (Table 3).

If Cu deficiency for cattle is defined as <5 ppm for ruminants [26] and the MTL of 40 ppm of Cu in the diet is harmful to cattle, then the number of samples greater than MTL of 40 ppm for cattle was 56 (15 percent, high), and the number of the 376 samples <5 ppm was 21 (6 percent, deficient). The maximum amount of Cu in the finished feed category for beef cattle was 371 ppm from poultry litter used as a source of non-protein nitrogen (Table 3). Cu concentrations were unexpectedly high in liquid feed for cattle, with a maximum concentration of 98 ppm (Table 3). Similar to cattle, assuming that Cu deficiency for sheep is defined as <5 ppm for ruminants [26] and the MTL of 15 ppm of Cu in the diet is harmful to sheep, then the number of the 41 samples greater than the MTL of 15 ppm for sheep was 19 (46 percent), and the number of samples <5 ppm was 1 (2 percent) (Table 3). There was a high frequency (45 percent) of excess Cu greater than the MTL of 15 ppm, and a low frequency (<5 ppm, 1 percent) of Cu deficiency in the finished feed category for sheep and goats.

3.5. Molybdenum in finished feeds

The ratios of the mean Mo concentrations in the nine finished feed categories, compared to the MTL, ranged from 1 to 37 percent (Table 3) for all species. The relatively low ratios of the mean Mo concentrations, compared to the MTL, for the four finished feed categories for cattle, sheep, and goats ranged from 14 to 25 percent (Table 3). This suggests an inadequate amount of Mo in the ruminant diets to antagonize, or counterbalance, the high dietary Cu. The number of samples greater than the Mo MTL of 5 ppm for cattle and sheep was zero. Assuming that Mo deficiency for ruminants is <2 ppm in the diet, to account for the ideal Cu:Mo ratio of >6:1 and <10:1, the number of samples <2 ppm for cattle was 339 (91 percent), and the number of samples >2 ppm was 31 (9 percent). For sheep and goats, the number of samples <2 ppm was 37 (90 percent), and the number of samples >2 ppm was 4 (10 percent). This suggests that the four finished feed categories for the three ruminant species contain inadequate amounts of Mo (90 percent, <2ppm) to antagonize the high Cu in the diet.

3.6. Copper:molybdenum ratio

High Cu and low Mo in the four finished feed categories points to an imbalance of Cu and Mo in ruminant diets (Tables 1-3 and Figure 2); the calculation of the overall Cu:Mo ratio of 26:1 for ruminants shows that imbalance (Figure 2). Although liquid feed supplement is not a sole source of nutrition for cattle, the high Cu:Mo ratio of 87:1 for liquid feed contributes to the high overall Cu:Mo ratio of 26:1. The Cu:Mo ratios for overall, beef cattle, dairy cattle, liquid, and sheep and goats were 2.6, 2.6, 3.1, 8.7, and 1.4 times, respectively, greater than the maximum ideal Cu:Mo ratio of <10:1. Therefore, there is an excess of Cu in ruminant diets, especially in sheep diets, exacerbated by inadequate dietary Mo.

Finished Feed Category	Mean (ppm)	SD [a]	Maximum (ppm)	MTL [b]	Ratio (%) [c]
Copper					
Liquid [d]	61.07	29.61	98	40	153
Sheep and Goat	18.29	17.36	113	15	122
Wildlife [e]	40.42	23.18	79	40 [e]	101
Beef Cattle	28.45	47.98	371	40	71
Dairy Cattle	25.69	16.61	70	40	64
Swine	67.81	84.34	282	250	27
Miscellaneous [f]	35.35	40.87	146	250 [f]	14
Horse	25.77	25.52	117	250	10
Poultry	19.49	11.24	70	250	8
Molybdenum					
Wildlife [e]	1.86	0.62	3.00	5 [e]	37
Horse	1.56	0.83	2.90	5	31
Sheep and Goat	1.27	0.77	4.20	5	25
Beef Cattle	1.12	0.81	3.80	5	22
Dairy Cattle	0.84	0.70	2.60	5	17
Liquid [d]	0.70	0.28	1.20	5	14
Poultry	1.71	0.83	3.50	100	2
Miscellaneous [f]	1.53	0.76	2.70	150 [f]	1
Swine	1.45	0.68	3.80	150	1

[a]SD = Standard deviation

[b]MTL = Maximum tolerable level of Cu and Mo [26]

[c]Cu/MTL Ratio = Mean Cu concentration in nine finished feed categories divided by Cu MTL x 100%; Mo/MTL Ratio = mean Mo concentration in nine finished feed categories divided by MTL for Mo x 100%

[d]Liquid feed is for cattle.

[e]Wildlife feed is mainly for deer. The MTL is estimated for deer based on the scientific litera-tureand the MTL for cattle.

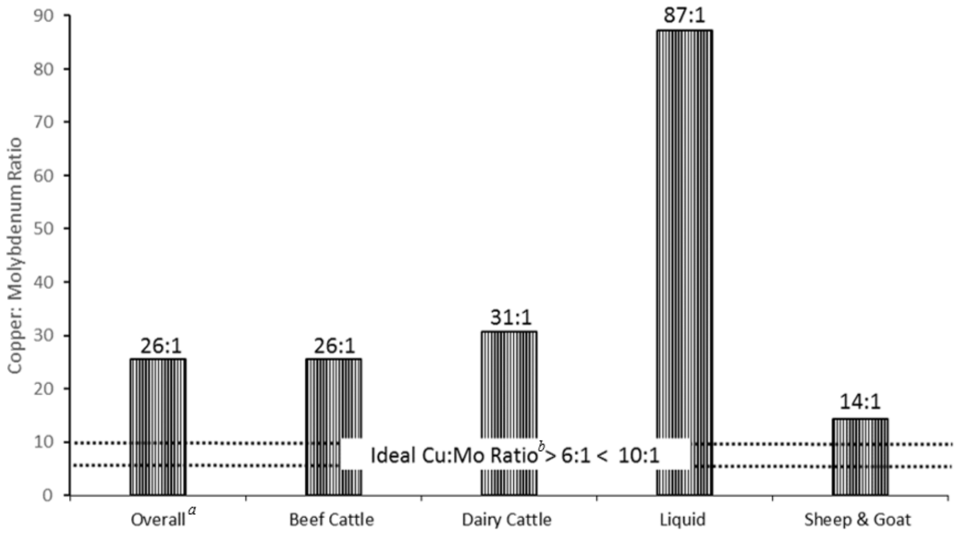
[f]Dog and cat food was incorporated into the miscellaneous feed category. There are no MTL set by the National Research Council for dog and cat food. A Cu MTL of 250 ppm and a Mo MTL of 150 ppm are reasonable estimates for dogs and cats as monogastric species.

Table 3: Ratio of the mean concentrations of copper (Cu) and molybdenum (Mo) in nine finished feed categories.

4. Discussion

The risk estimates for the six contaminants range from 2 to 4, compared to a maximum value of 12, which indicates no or low risk of toxicity to animals exposed to these contaminants that are potentially carried over from feed ingredients to finished feeds. Although this is not unequivocal proof that there is negligible carryover of the six contaminants from feed ingredients to finished feeds, it provides compelling evidence based on more than 500 samples for each contaminant. As a worst-case scenario, if the mean plus 3 SD for each of the six contaminants is calculated across 11 finished feed categories, the calculated value is still well below the MTL of the respective contaminant. Assuming a normal distribution, the mean concentration plus 3 SD above and below the mean concentration represents 99 percent of the data or 99 percent of the data is below the maximum concentration and MTL of each mineral. For example, the mean concentration of 0.28 ppm plus 3 SD for arsenic across 11 finished feed categories, 0.86 ppm, which is below the maximum concentration of 3.5 ppm in finished feed (Table 1) and about 36 times less than the MTL of 30 ppm for cattle [26]. Thus, the six contaminants are unlikely to be harmful to animals based on the calculation of the 99th percentile as the mean concentration plus 3 SD.

There is a narrow margin of safety for Se between nutritional requirements and toxicity [26]. It is unknown whether the high Se in the seven samples from the beef cattle category was due to a sporadic mixing error of mineral mixes with no adverse effects. Possibly, the high Se could have originated from exogenous sources. In fact, six of the nine finished feed categories had a mean Se concentration below the LOD of <1 ppm. Se requirements of various species, including fish, fall between



[a]Overall ratio = Mean Cu concentration divided by mean Mo concentration across the 4 finished feed categories
[b]Cu:Mo ratio = Mean Cu concentration divided by mean Mo concentration

Figure 2: Copper:molybdenum (Cu:Mo) ratios are calculated from four finished feed categories for beef cattle, dairy cattle, liquid feed (cattle), and sheep and goats. The ideal Cu:Mo ratio for ruminants (cattle, sheep, and goats) is >6:1 and <10:1.

0.1 and 0.38 mg/kg of diet [24]. Therefore, the mean concentrations of Se in nine finished feed categories are adequate, with some concern for toxic amounts in beef cattle feed.

The high Cu concentrations in finished feeds imply that there is a likely risk of Cu toxicosis for ruminants with long-term exposure. In general, domestic ruminants are more susceptible to Cu poisoning than monogastrics [26]. Cu requirements in ruminants are generally greater than in non-ruminants, and vary from approximately 4 to 20 mg Cu/kg of diet, depending on the dietary concentrations of Cu antagonists, such as Mo and sulfur (S) [32, 26]. Relatively high dietary concentrations of Mo and S increase the Cu requirements by two to three times. Non-ruminants require about 4 to 8 mg Cu/kg diet as a growth promotant [32, 26]. In non-ruminants, Cu homeostatic control mechanisms are very efficient in preventing toxicosis. Concentrations of Cu needed to cause toxicosis in non-ruminants exceed requirements by at least 25 times and are as high as 50 times greater in pigs [6]. In contrast, long-term exposure to <40 ppm Cu in the diet caused death in nine out of 63 dairy cattle that received a diet containing 37.5 mg Cu/kg of dry matter (DM) during lactation, and 22.6 mg/kg of DM during the dry period for over two years [5]. Thus, even a "safe" amount of Cu in the diet can cause toxicosis. A copper MTL of 25 ppm for cattle may be more appropriate than the 40 ppm MTL, because 124 (33 percent) of the 376 samples had a level greater than 25 ppm.

The high concentration of Cu in the finished feed category for sheep and goats is noteworthy, due to the inherent sensitivity of sheep to Cu toxicosis. Death due to Cu toxicosis is a common problem in sheep [26]. The sensitivity of sheep to Cu toxicity relates to their inability to increase biliary Cu excretion in response to high intakes [6]. Goats are less sensitive than sheep, but more sensitive than cattle to Cu toxicity [26]. The range of dietary Cu concentrations required by sheep under some conditions can overlap with dietary concentrations that cause toxicosis under other conditions. For example, 10 mg Cu/kg diet may be required by sheep if dietary S and Mo are >0.3 percent of DM and >2 mg/kg of DM, respectively [26]. If dietary Mo is low at <1 mg/kg of DM, 10 mg Cu/kg diet can cause toxicosis in some breeds of sheep [14]. Mo in ruminant diets is frequently within the range of 1 to 5 mg Mo/kg DM, while total S varies from 0.1 to 0.3 percent of DM [26]. Concentrations of Mo and S on the upper end of these ranges reduce Cu bioavailability and increase the risk of Cu deficiency. Conversely, low dietary concentrations of Mo and S increase the risk of Cu toxicosis, especially in sheep. Cattle and goats are less susceptible to Cu toxicity than sheep. Therefore, the data in this report support that sheep are especially vulnerable to Cu toxicity at concentrations >15 ppm in sheep and goat feed with low Mo.

The nutritional balance of Cu and Mo in the ruminant diets goes hand in hand, so that the deficiency of one causes the toxicity of the other. The low Mo concentrations in finished feeds for ruminants imply that the risk of Cu toxicosis is increased with long-term exposure. The absorption of Mo across the mucosa is an active, carrier-mediated process in the intestine (absorption) and kidney (reabsorption) that antagonizes the absorption of Cu and sulfate salts (sulfur) [26, 19]. This interaction explains why increasing dietary S decreases absorption or retention of Mo in ruminants [9, 23]. Mo requirements for goats, rats, chicks, and other species are <0.2 mg/kg of diet [20] and Mo deficiency is rare in animals fed nutritionally adequate diets [26]. However, the nutritional requirements do not consider the ideal Cu:Mo ratio of >6:1 and <10:1 for ruminants [21]. If the ideal Cu:Mo ratio is <10:1, and the Mo concentration is nutritionally adequate at <0.2 mg/kg of diet, the corresponding Cu concentration is approximately 2 mg/kg of diet, resulting in a net Cu deficiency for ruminants. This would not make sense, because ruminants require about 5 to 10 times more than the 2 mg Cu/kg of diet. Perhaps a more realistic but hypothetical Mo concentration for ruminant feeds would be approximately 2 mg Mo/kg of diet instead of 0.2 mg. Logically, Mo "deficiency" for ruminants should be redefined as <2 ppm in the diet, to account for the ideal Cu:Mo ratio of >6:1 and <10:1. The corresponding Cu concentrations should be about 20 mg/kg of diet, with a Cu:Mo ratio of about 10:1 for cattle, and about 12 mg/kg of diet with a Cu:Mo ratio of about 6:1 for sheep. These dietary Cu concentrations would be within the MTL of 40 ppm and a proposed MTL of 25 ppm for cattle, and the MTL of 15 ppm for sheep.

Adding more Mo to ruminant diets is not an option because Mo is not an approved food additive. However, the AAFCO Official Publication states that it is generally recognized that Mo may be added to sheep feed at 1 to 3 ppm [2]. There also may be Mo from unknown sources such as water, to antagonize the high Cu in four finished feed categories for ruminants, but it is not likely. Another approach would be to decrease the amount of Cu in ruminant diets, but this should be done with caution to avoid Cu deficiencies. To that end, it should be kept in mind that the Cu bioavailability in the four finished feed categories for ruminants may be lower than expected. Cu absorption in ruminants is low, at 1.0 to 10.0 percent, relative to monogastrics [29]. Copper oxide is insoluble and is not absorbed even under acidic conditions in the abomasum. The low absorption of Cu in ruminants relative to monogastrics is mainly a result of the complex interactions in the rumen environment. Tribasic cupric chloride is more soluble than cupric sulfate in the rumen environment of cattle with high amounts of Mo and S [29]. The high bioavailability of Cu from tribasic copper chloride compared to copper chloride may relate to low solubility in the rumen and the interference of Mo and S.

Typical diets for ruminants contain about 0.2 percent S [26]. Exogenous sources of dietary S, such as high residual S (0.45 to 0.85 percent) in corn byproducts derived from ethanol production, antagonize Cu absorption [26, 25]. However, high dietary S (>0.4 percent of diet) can produce polioencephalomalacia, a debilitating brain disease in ruminant [26]. The possibility of polioencephalomalacia in ruminants may motivate producers and feed manufacturers to restrict dietary S to 0.2 to 0.3 percent of DM. Thereby, Cu absorption is increased, resulting in a greater likelihood of Cu toxicity from ruminant diets with Mo <2 ppm, Cu >15 ppm for sheep and >25 ppm for cattle, and a Cu:Mo ratio >10:1. Regardless of Cu bioavailability and Mo and S as Cu antagonists in the ruminant diets, the absence of corroborating reports of Cu toxicosis in the scientific literature may indicate that ruminants are not being exposed to toxic amounts of dietary Cu. Alternatively, Cu toxicosis in ruminants may be underreported, especially in show sheep that are often fed diets that are formulated with excess Cu.

5. Conclusion

The potential carryover of the As, Cd, Pb, Hg, Ni, and Tl from feed ingredients to finished feeds is not likely to harm animals. The low risk of exposure is corroborated by the low mean concentrations of the six contaminants in finished feeds. The mean concentrations of Cr and Se as essential minerals in finished feeds are nutritionally adequate with a low likelihood of causing toxicity. A high overall Cu:Mo ratio of 26:1 indicates a nutritional imbalance of Cu excess and Mo deficiency in finished feeds for ruminants. Perhaps a more appropriate Cu MTL for cattle should be 25 ppm rather than 40 ppm, to prevent excess Cu in ruminant diets, but would require adoption by the NRC. Mo could be increased in ruminant diets to partially offset excess Cu, but it may not be practical due to regulatory limitations. Cu could be easily reduced in ruminant diets, but it should be done with caution to prevent Cu deficiency. In addition to reducing dietary Cu, the effects of other mitigating factors, such as low Cu bioavailability and S antagonism, are unknown and could cause a net Cu deficiency. Other state, federal, and academic entities are encouraged to build on this risk assessment by publishing their findings in the Journal of Regulatory Science for mineral contaminants and essential macro and micro minerals in animal food/feed.

6. Declaration of Conflicting Interest

The authors declare no conflict of interest.

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