Appendix B

Copper in Dog Foods Expert Panel
Final Report with Recommendations to the Pet Food Committee

Introduction

The Expert Panel (formed in accordance with the Criteria for Nutritional Indicators in the AAFCO Official Publication at the request of the Pet Food Committee) met four times (on May 4, June 7, and July 23, 2021 and finally on July 12, 2022) to consider the requests made in a Viewpoint article published in the February 15, 2021 edition of the *Journal of the American Veterinary Medical Association* by Dr. Sharon Center *et al*. titled *Is it time to reconsider current guidelines for copper content in commercial dog foods?* (Viewpoint Article)[1] and what empirical scientific findings could be used to evaluate and definitively address the issue. After the January 2022 mid-year AAFCO meeting, three additional members were solicited and agreed to serve on the Expert Panel, Dr. Joseph Wakshlag DVM, PhD, DACVSMR, DACVIM(nutrition) at Cornell, Dr. Andrea Fascetti, VMD, PhD, DACVIM(internal medicine, nutrition) at U. C. Davis, and Dr. George Collings, CEO & President of Pet Solutions Group all of whom were present and provided input at the meeting on July 12, 2022. Members of the final Expert Panel, all present at the July 12, 2022 meeting, were:

Dr. William J. Burkholder, Expert Panel Chair, PFC Member, CVM/DAFI Employee
Dr. Andrea Fascetti, University of California Davis
Dr. Angele Thompson, Consultant, Thompson Pet Tech, PFI Representative
Ms. Charlotte Conway, PFC Member, CVM/DAFI Employee
Dr. Dana Tomlinson, Zinpro, AFIA Representative
Dr. Dave Dzanis, Consultant, APPA Representative
Dr. Gail Czarnecki-Mauldin, Nestle Purina
Dr. George Collings, Pet Solutions Group, Consultant
Dr. George Fahey, Jr. University Illinois, PFI Representative
Dr. Joseph Wakshlag, Cornell University
Dr. Karen Donnelly, CVM/DAFI Employee
Dr. Laura Amundson, Zinpro, Alternate AFIA Representative
Ms. Louise Calderwood, AFIA
Ms. Madison Fink, Missouri Department of Agriculture, PFC Member, Project Manager

Findings

The Panel had the summary document for the first three meetings (Attachment 2 to this report) and a document from Zinpro about copper requirements and the bioavailability of ingredients used to supplement copper to diets (Attachment 3 to this report) available to the members one month prior to the meeting on July 12, 2022. Members of the Panel were asked to provide any additional critical information from the scientific literature required to make an informed decision for recommendations to the Pet Food Committee (PFC) that was missing from the summary document or bioavailability document. No specific publications were brought forward.

[1] Center SA, Richter KP, Twedt DC, Wakshlag JJ, Watson PJ, Webster CRL. Is it time to reconsider current guidelines for copper content in commercial dog foods? *J. Am. Vet. Med. Asso.* 258(4): 357-364, 2021.

On the question/request in the Viewpoint Article to restrict the source for adding copper to dog foods to copper oxide (cupric oxide), the majority of the Panel expressed opposition to such a restriction noting that the bioavailability of cupric oxide was essentially zero and thus would add nothing of value to the dietary formula.

Some discussion occurred concerning adding a footnote to the AAFCO Dog Food Nutrient Profiles to suggest that sources of chelated copper be restricted to approximately 25% of the added copper in diets with the remaining added copper being supplied from inorganic mineral salts. One member of the Panel felt that this was a widely used rule-of-thumb when chelated sources of copper first came on the market that formulators have perhaps forgotten. However, the Panel did not express an overwhelming position that such a footnote was needed. More discussion about this aspect might be undertaken at the request of the PFC.

The Panel had previously rejected decreasing the recommended amount of copper to 3.6 mg Cu/kg DM (0.9 mg Cu/1000 kcal metabolizable energy) as this amount of copper is less than the amounts for adequate intake or recommended allowances for any life stage of dog established by the National Academies of Sciences' 2006 Nutrient Requirements of Dogs and Cats Expert Subcommittee and would run the risk of producing a copper deficiency especially in gestating and lactating female dogs.

The Expert Panel struggled with setting a maximum amount of copper in the AAFCO Dog Food Nutrient Profiles for the following reasons:

1. As determined by the National Academies of Sciences' 2006 Nutrient Requirements of Dogs and Cats Expert Subcommittee, there is insufficient empirical data to establish a safe upper limit or maximum tolerable level in normal dogs. As evidenced by the information in the Attachment 2, this continues to be the condition in 2022. No additional empirical data has been added to the scientific nutritional literature that bears on what a maximum limit for copper should be in normal dogs in the years since 2006.

2. Because of number 1., setting any value for a maximum amount of copper in complete diets for dogs would simply be an arbitrary decision, not based on science.

3. Furthermore, arbitrarily setting some value as a maximum for copper implies that diets containing less than, or equal to, the maximum are safe for dogs and that diets containing more than the maximum amount are unsafe, with neither condition having been demonstrated to be true.

For these reasons the majority of the Panel does not believe it is possible to set a maximum amount for copper in complete diets for dogs. More *in vivo* work is required to establish what that maximum value is. Until such studies are completed and have passed peer review, setting a value will have the consequences outlined in #3 above. The Panel notes the studies must be done using members of the target species (i.e., dogs) but that such studies need not necessarily be terminal. However, the consequences of the current criticism and condemnation of *in vivo* studies in companion animals makes it unlikely that the necessary work for setting a maximum amount of copper in normal dogs will be accomplished any time soon.

However, the majority opinion, that no maximum amount of copper should be set for complete diets for dogs until objective scientific data is available to establish such a maximum, was not unanimous among the Expert Panel members. Two Panel members still felt that some maximum amount should be set despite having heard the reasoning and discussion resulting in numbers 2 and 3 above. One member indicated to the chair, upon review of the draft of this report, that a maximum of between 30 and 40 ppm of copper on a dry matter basis should be set by AAFCO. The other member, again upon review of the draft of this report, indicated to the chair that they felt that 25 ppm copper on a dry matter basis should be the maximum. Twenty-five ppm is the amount of copper the European Union has set as a maximum for copper in dog foods based on environmental concerns and this maximum has been incorporated by the Federation Europenne de l'Industrie des Alimenttis pour Animaux Familiers (FEDIAF) into their nutrient profiles.

The Panel does understand the concern some people may have for knowing the amount of copper in their dog's diet and for wishing to feed a "low copper" formula. As an alternative to setting a maximum copper content for dog foods, the Panel briefly discussed and suggests that the PFC consider establishing a regulation under the Descriptive Terms Model Regulation PF10 for when a dog food may make a claim to be low in copper,

The Expert Panel felt that the upper limit for dog foods making a 'Low Copper' claim would be 15 mg Cu/kg dry matter and 3.75 mg Cu/1000 kcal of metabolizable energy based on the measured amounts of copper in dog food products observed between 2017 and 2021 (see Figures 1 and 2 in Attachment 2). Also, the Panel discussed the likelihood that no mammal needs more than 15 mg Cu/kg dry matter under normal circumstances to meet nutritional requirements. In support of the suggestion for a regulation providing for a low copper claim on dog foods, this report includes a draft of the language to place in Model Regulation PF10 as Attachment 1. There are three critical components to the criteria that the food must meet in order to bear a 'Low Copper' claim:

1. As already stated, the food must contain no more than 15 mg Cu/kg dry matter and no more than 3.75 mg Cu/1000 kcal of metabolizable energy. The PFC should note that this claim is tied to an absolute quantity of copper, not a relative amount; and

2. The food must be complete and balanced according to Model Regulation PF7. This means that unless the formula undergoes and passes the appropriate AAFCO Feeding Protocol for the life stage of the dog, the copper content of the 'Low Copper' diet must be between the minimum amount indicated for the life stage in the AAFCO Dog Food Nutrient Profiles and the maximum of 15 mg Cu/kg dry matter and no more than 3.75 mg Cu/1000 kcal of metabolizable energy; and

3. The product label must bear a guarantee for the maximum amount of copper in the product in the Guaranteed Analysis according to Model Regulation PF4.

The Panel strongly recommends that if this proposed model regulation is pursued, the PFC should ensure these 3 criteria remain intact if and when any draft language moves forward through the reviewing committees, the AAFCO Board and the membership for approval. These criteria ensure that: 1) the foods bearing the claim are complete and balanced for normal dogs; 2)

the copper content will be less than the concentration of copper in the majority of commercial products currently on the market (see Figures 1 and 2 in Attachment 2); and, 3) the dog owner will be informed as to the maximum content of copper contained in the product bearing the 'Low Copper' claim.

Although the minimum and maximum copper contents of products bearing a 'Low Copper' claim would be greater than the minimum and maximum copper content proposed in the Viewpoint Article for all dog food products, the dogs, seen by veterinary internists and nutritionists at teaching institutions such as the authors' of the Viewpoint Article, are ostensibly dogs with a disease, likely resulting from a genetic abnormality in the coding for one or more proteins involved in the normal copper clearance mechanism present in normal dogs. The copper content proposed for all dog foods by the authors of the Viewpoint Article would make the products therapeutic products that should be used under the supervision of a licensed veterinarian as provided for by the AAFCO Guidelines for Making Therapeutic Diet Claims and the FDA Compliance Policy Guide 690.150. As indicated above, the majority of the Expert Panel does not believe such restrictive amounts of copper to be appropriate for generally available commercial dog food products.

Conclusion

The AAFCO Pet Food Committee Copper in Dog Foods Expert Panel recommends that the AAFCO Pet Food Committee:

1) Not pursue a restriction for allowing only copper oxide as the form of copper allowed for copper supplementation of dog foods;
2) Not establish a maximum for the overall copper content of dog foods within the AAFCO Dog Food Nutrient Profiles;
3) Consider and further explore establishing within Model Regulation PF10 Descriptive Terms the criteria for commercial dog food products to bear a 'Low Copper' claim, as provided for in the language in Attachment 1 to this Report; and,
4) Disband this Expert Panel.

Respectively submitted to the AAFCO Pet Food Committee this 1st day of August, 2022.

William J. Burkholder, DVM, PhD, DACVIM(Nutrition)
Chair
AAFCO Pet Food Committee Copper in Dog Foods Expert Panel

Copper in Dog Foods Expert Panel
Final Report with Recommendations to the Pet Food Committee

Attachment 1

Regulation PF10. Descriptive Terms
[…]
(d) Low Copper
A dog food that bears on its label the claim "low copper," "low in copper," or words of similar designation shall:

(1) Be substantiated as nutritionally adequate for one or more life stages in accordance with Regulation PF7; and
(2) Contain a maximum of no more than 15 mg copper/kg DM and no more than 3.75 mg copper/1000 kcal of metabolizable energy; and
(3) Bear on its label in the Guaranteed Analysis in accordance with Regulation PF4 a guarantee for the maximum amount of copper in the dog food.

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Copper in Dog Foods Expert Panel
Final Report with Recommendations to the Pet Food Committee

Attachment 2

Summary and Status of Copper in Dog Foods Work Group as of June 6, 2022

Introduction

The work group (WG) met three times (on May 4, June 7, and July 23, 2021) to consider the requests made in a Viewpoint article published in the February 15, 2021 edition of the *Journal of the American Veterinary Medical Association* (JAVMA) by Dr. Sharon Center *et al*. titled *Is it time to reconsider current guidelines for copper content in commercial dog foods?*[1] and what empirical scientific findings could be used to evaluate and definitively address the issue. After the January 2022 mid-year AAFCO meeting, three additional members were solicited and agreed to serve on the work group, Dr. Joseph Wakshlag DVM, PhD, DACVSMR, DACVIM (nutrition) at Cornell, Dr. Andrea Fascetti, VMD, PhD, DACVIM (internal medicine, nutrition) at U. C. Davis, and Dr. George Collings, CEO & President of Pet Solutions Group.

In the JAVMA Viewpoint article, the authors contend that both the digestibility (bioavailability) and the amount of ingredients, other than copper oxide, used to supplement copper into dog foods have caused the copper content in the liver of dogs to steadily increase over time to where the copper content of canine livers is now significantly greater than it was prior to some reference time point within the last 10 to 25 years. That copper concentrations observed in the liver of dogs have increased over the last 10 to 25 years is well documented by multiple independent investigators and publications.[2,3] (See also Appendix 1 and its associated references.) Whether this increase is a result of the methods used to quantify liver copper concentrations,[4,5] the number of samples being analyzed within a given period of time, a change in genetic predispositions within the general dog population,[6-9] or the copper content and composition of dog foods is unclear. Some researchers believe commercial dog food to be the predominant cause,[1,10] but others do not.[11] The lack of control for, and/or the quantitative composition of, each of these factors makes the proportional contribution of each factor hard or impossible to independently assess, but it is likely to be a combination of all factors mentioned.

Copper Restriction Proposed by Dr. Center *et al.*

In the JAVMA Viewpoint article, Dr. Center *et al*. proposed that supplemental copper (Cu) in dog foods be restricted to copper oxide in the range of 0.9 mg Cu/1000 Kilocalories metabolizable energy (Kcal ME) to 1.1 mg Cu/1000 Kcal ME (equivalent to 3.6 mg Cu/kg dry matter (DM) to 4.4 mg Cu/kg DM in a diet containing 4000 Kcal ME/kg DM).[1]

The WG feels there are many problems with this proposed range for the Cu content of dog foods. First, such a limited restriction is generally not required for foods, even for nutrients such as iodine and selenium with known toxicities within a limited range between nutritional adequacy and toxic amounts. A 0.2 mg Cu/1000 Kcal ME range between minimum and maximum amounts would indicate that copper is more toxic to dogs than selenium (Se), another trace element that can produce toxicity in excessive amounts, but which is nutritionally tolerated within the range of 0.3 – 2.0 mg Se/kg DM (0.075 – 0.5 mg Se/1000 Kcal ME). Suffice it to say

that nutritional science has not reached a consensus that Cu is more toxic than Se. Furthermore, the maximum amount of 1.1 mg Cu/1000 kcal ME is less than the smallest amounts for Adequate Intakes or Recommended Allowances for dogs set by the National Academies of Sciences' 2006 Nutrient Requirements of Dogs and Cats Expert Subcommittee of 1.5 mg Cu/1000 Kcal ME.[12]

The WG believes that setting the Cu recommended and maximum amounts between 0.9 to 1.1 mg Cu/1000 Kcal ME makes the occurrence of a Cu-deficiency more likely, particularly for reproducing female dogs with a recommended Adequate Intake (AI) of 3.1 mg Cu/1000 Kcal ME,[13] despite the contention by Dr. Center *et al*. that Cu-deficiency has not been observed in dogs even when using copper oxide as the primary source of supplemental copper. Finally, no consideration for the content of other trace minerals in the diet that are antagonistic to the uptake of Cu, such as zinc or iron, has been made when recommending the range of 0.9 to 1.1 mg Cu/1000 Kcal ME for dog foods. This could lead to a Cu-deficiency or perhaps a toxicity of one of the competitive minerals.

Is Copper Restriction Needed?

Empirical Evidence

The National Academies' *Ad Hoc* Committee on Dog and Cat Nutrition did not elect to set a maximum Safe Upper Limit (SUL) for Cu content of dog foods when it revised the nutrient requirements for dogs and cats in 2006, stating there was lack of sufficient data to set such a value. In consideration of this result the AAFCO Canine Nutrition Expert Subcommittee withdrew the previous recommended maximum of 300 mg Cu/kg DM (75 mg Cu/1000 Kcal ME) from the AAFCO Dog Food Nutrient Profiles that was an extrapolation from the maximum tolerance for swine.[14]

In reviewing the scientific literature for what might be considered a maximum recommended or safe upper limit for Cu in dog foods based on empirical data, the current WG made note of two studies. One was the lifetime feeding study of Labrador Retrievers performed by Ralston Purina in the late 1980's through the 1990's where the dogs were fed a diet containing 12.3 mg Cu/kg DM (~ 3-4 mg Cu/1000 Kcal ME) with Cu supplemented in the diet by copper sulfate.[15] The dogs in this study were followed throughout their lives until they died with none being reported of having died due to a hepatopathy. Dr. Center has commented in discussions with people in the Center for Veterinary Medicine (CVM) that she has seen sections of liver from the dogs on the Purina study and that the sections contained copper. However, this is not surprising as the liver is known to be the storage organ for copper. Thus, the question is not whether copper was present in the liver of the dogs, but rather what the overall histologic appearance of the liver sections and the concentration of copper in the liver was. Whatever those conditions were, they were evidently consistent with normal liver function for Labrador Retrievers throughout their lives until death at 12–13 years of age.

The WG also made note of a Research Report for a study done in 1972 at the Warner-Lambert Research Institute by J. E. Shanaman and colleagues,[16] some details of which were summarized in the publication *Environmental Health Criteria 200 Copper* in 1998.[17] In this study, young (still growing) Beagle dogs were fed specific amounts of copper gluconate as part of their diet

for 6-12 months. There were 4 groups of 6-8 male and 6-8 female Beagles per group. Group 1 (Control) received no Cu-gluconate in their diet. Groups 2, 3 and 4 received diets containing 0.012%, 0.06% and 0.24% Cu-gluconate that provided 0.42, 2.1 and 8.4 mg Cu/kg body weight/day (kg BW/d), respectively. Dogs underwent physical examinations and evaluations of hematologic, biochemical, and urologic parameters during and at the end of the study, as well as necropsies and histopathological examination of tissues at the end of the study.

There were no abnormal findings on the basis of physical examinations, weight gain, hematologic or urologic evaluations during the study or at study completion. Two dogs fed 8.4 mg Cu/kg BW/d had increased concentrations of serum glutamic-pyruvic transaminase (SGPT, a liver enzyme) which was reversible on removal from the diet. At 6 and 12 months there was an increase in tissue Cu concentrations of the liver, kidney and spleen associated with dietary intakes, although the specific tissue concentrations were not reported in current literature. The study investigators concluded that the increased SGPT was not of toxicological significance, but this conclusion is not in line with current veterinary interpretation of such a result, particularly in the absence of liver copper concentrations for the dogs eating 8.4 mg Cu/kg BW/d. One might justifiably conclude based on what is known from the study that dogs could safely consume 2.1 mg Cu/kg BW/d for one year, but that 8.4 mg Cu/kg BW/d is above the no observed adverse effect level (NOAEL) for dogs based on the finding of increased SGPT in 2 dogs in the study group consuming the most Cu/kg BW/d. This conclusion might change if the tissue concentrations were known for the various groups, depending on their magnitudes.

An estimation for the amount of Cu/kg DM of diet and the amount of Cu/1000 Kcal ME in the diets fed in the Shanaman *et al*. study can be made based on assumptions that:

- Adult Beagle Dogs weigh between 9.09 and 13.63 kg (20-30 lbs.).[18]
- The energy requirement for average laboratory dogs is given by $130((BW_{kg})^{0.75})$.[19]
- The diet contained 4000 Kcal ME/kg DM.

Table 1 Estimate of Copper Concentrations of Diets Used in the Study by Shanaman *et al*. Evaluating the Chronic Oral Toxicity of Copper Gluconate

	Min.	Max.	
Range of BW for Normal Beagle Dogs (kg)	9.09	13.63	
Amount of Cu @ 2.1 mg Cu/kg BW/d (mg)	19.09	28.62	No Adverse Effects
Amount of Cu @ 8.4 mg Cu/kg BW/d (mg)	76.36	114.49	Adverse Effects
Range of Calories Consumed/day (Kcal ME)	681	922	
Cu @ 2.1 mg Cu/kg BW/d (mg/1000 Kcal ME)	28	31	
Cu @ 8.4 mg Cu/kg BW/d (mg/1000 Kcal ME)	112	124	
Cu @ 2.1 mg Cu/kg BW/d (mg/kg DM)	112	124	
Cu @ 8.4 mg Cu/kg BW/d (mg/kg DM)	448	496	

28 mg Cu/1000 Kcal ME < NOAEL < 124 mg Cu/1000 kcal ME
112 mg Cu/kg DM < NOAEL < 496 mg Cu/kg DM

As shown in Table 1, it might be concluded from the known results of the study by Shanaman *et al*. that the dogs in the group consuming 2.1 mg Cu/kg BW/d ate amounts of Cu equal to or less than the NOAEL for dietary Cu, so 28-31 mg Cu/1000 Kcal ME (112-124 mg/kg DM) should be equal to or less than the NOAEL or SUL for consumption of Cu for at least a year by dogs; whereas, 112-124 mg Cu/1000 Kcal ME (448-496 mg Cu/kg DM) is greater than the NOAEL for dietary copper in dog foods fed for one year. However, this conclusion may be influenced or altered if the tissue concentrations of Cu in the dogs from the Shanaman *et al*. study were reported in the summarized results of the study, which they were not, and thus, the concentrations of Cu in the tissues are not generally known at this time.

The range between ~30 mg Cu/1000 Kcal ME and ~120 mg Cu/1000 Kcal ME is broad and an estimate of what the precise NOAEL for Cu content is cannot be determined based on the data available. It is for this reason, lack of sufficient data to set a definitive upper limit for Cu, that the National Academies' *Ad Hoc* Committee on Dog and Cat Nutrition did not elect to set a SUL and the AAFCO Canine Nutrition Expert Subcommittee withdrew the previous recommended maximum of 75 mg Cu/1000 Kcal ME (300 mg Cu/kg DM) from the AAFCO Dog Food Nutrient Profiles.

Perspectives from Comparative Animal Nutrition

Sheep are known to be a domestic species sensitive to Cu in excess of 15 mg Cu/kg DM, dependent on the dietary content of competitive minerals, particularly molybdenum, sulfur and iron, but also calcium and zinc.[20] The bioavailability of copper in traditional ovine diets is poor, ranging from 1.4 to 12.8%, and the bioavailability is stated to be more important than the Cu concentration in the feed.[21] Comparisons of other parameters concerning dietary Cu between dogs and sheep are unjustified because of the anatomical differences in digestive tracts and the different feedstuffs typically consumed by the two species. Suffice it to say that, at least until now, dogs have not been represented to be more, or even as, sensitive to dietary Cu than sheep.

To have attained a sensitivity to dietary Cu equal to, or greater than, that of sheep could indicate a substantial change in Cu metabolism and physiology of dogs likely due to genetic changes across the spectrum of different breeds of dogs, not solely within the so-called known predisposed breeds. The list of dog breeds with an identified or suspected genetic predisposition to Cu storage disease has expanded over the years, and now includes not only Bedlington Terriers, West Highland White Terriers and Labrador Retrievers but also Dobermans, and Dalmatians, and several other breeds and cross-breeds suspected based on histologic findings of Cu-associated hepatitis.[11] If expansion of in-bred abnormalities in genes affecting copper metabolism is in fact what has happened to the canine genome, then no data is currently available that would indicate what a life-time NOAEL is for Cu in such dogs and the limited data for setting minimum amounts of Cu in canine diets may be inapplicable to the new genome or at least the copper-sensitive genome(s) of dogs. Furthermore, the copper-sensitive genome would need to be shown to be the predominant genetic make up for the population of dogs, as nutrient requirements and tolerances are not usually set for minority abnormal genetic variants of a population.

As indicated for sheep, the bioavailability of copper in the food may be more important than the concentration of Cu in the food. There are very few studies assessing the digestibility (bioavailability) of mineral salts of Cu (Cu salts) or Cu chelates in dogs. Most estimates of

digestibility in other species provided as values relative (RV) to some reference standard that is a very available source of Cu such as cupric sulfate, cupric acetate or copper carbonate.[22] Data from other monogastric species (swine and rats) indicate the RVs for mineral salts range from 0 (cupric oxide) to near 100 (copper sulfate, copper carbonate) depending on the salt and the oxidation state of the Cu molecule being assessed relative to the reference standard. Values for organically bound Cu (Cu chelates) range from slightly greater than 100 to 247 in one study with most values being in the range of 125 to 150 (see Table 1 in Baker and Ammerman, Chapter 7 in Bioavailability of Nutrients for Animals, Amino Acids, Minerals, and Vitamins).[22]

This latter range of estimates for the bioavailability of Cu chelates may have led to the use of a rule of thumb among ration formulators for automatically reducing the amount of chelated ingredients by 25-75% relative to the amount of a Cu salt when substituting a Cu chelate for a Cu salt in a diet formula.[Collings, personal communication] Failure by ration formulators to account for the differences in bioavailability among Cu sources could lead to excess Cu supplementation and is one of the reasons Dr. Center and colleagues argue for a return to use of Cu oxide. **Thus, this rule of thumb should probably be archived as a footnote after the "copper oxide" footnote in the AAFCO Dog and Cat Food Nutrient Profiles, and the "copper oxide" footnote revised to indicate cupric oxide because cuprous oxide is estimated to be essentially equivalent to cupric sulfate at least in chickens.[22]**

Considerations for Setting a Maximum Recommended Amount of Cu in Dog Foods

Ten Times the Minimum Requirement – A Rough Maximum

A general rule of thumb among nutritionists is that approximately 10-times the minimum requirement is a reasonable maximum dietary content to adhere to if a definitive maximum has not been established and little is known about the concentration causing marginal toxicity. The absolute daily minimum requirement of Cu for dogs of any life stage has not been established, so "adequate intake" (AI) amounts are the smallest quantities determined for the Cu content of dog foods.[12] The AI concentration values and the values that are 10-times the AI concentrations for defined life stages of dogs are listed in Table 2.

Table 2. Adequate Intake Concentrations for Cu in Diets for Dogs of Various Life Stages and 10-Times Those Amounts

	mg Cu/1000 Kcal ME	
Life Stage	AI	10 x AI
Adult Maintenance	1.5	15
Growing Puppies	2.7	27
Late Gestation & Peak Lactation	3.1	31

	mg Cu/kg DM[a]	
Life Stage	AI	10 x AI
Adult Maintenance	6	60
Growing Puppies	11	110
Late Gestation & Peak Lactation	12.4	124

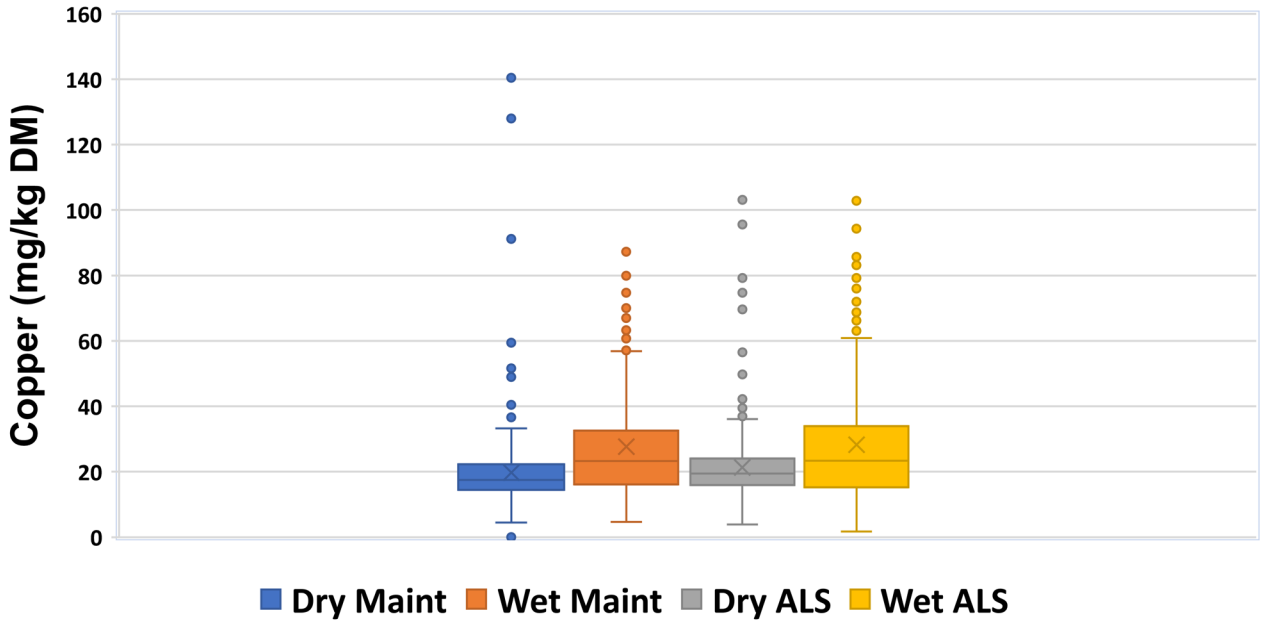
[a] Diets containing 4000 Kcal ME/kg DM

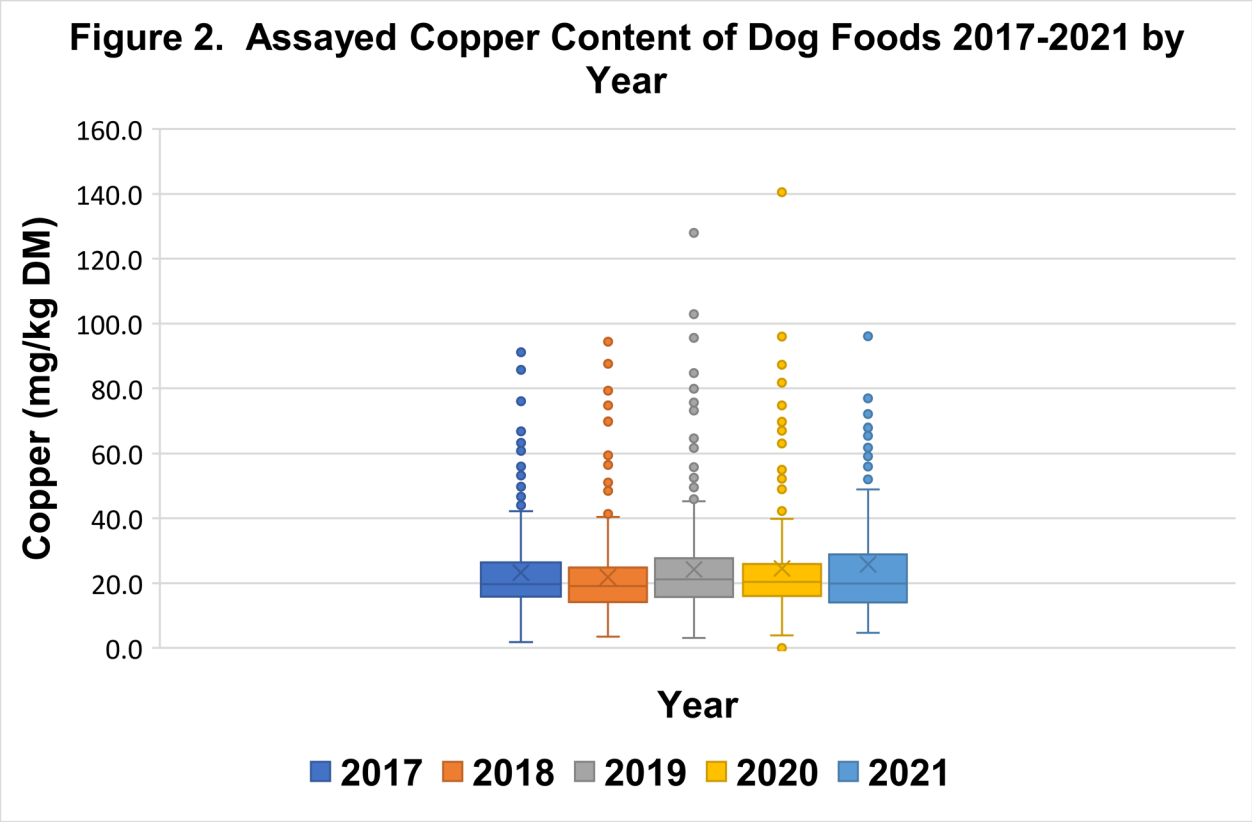
It may be worth noting that 10 times the AI value for late gestation and peak lactation agrees remarkably well with the values from the Shanaman *et al*. study for the group of Beagle dogs consuming 2.1 mg Cu/kg BW/d (Table 1). Still, the Cu concentrations in tissues produced by this amount of copper are unknown. Ten times the minimum requirement would place a maximum somewhere between 60 and 124 mg Cu/kg DM depending on which AI value is selected for the minimum amount. Although 10 times the AI would provide a rational basis for setting a maximum given the lack of definitive data, it is the author's opinion that a value of 60 to 124 mg Cu/kg DM is unlikely to offer any protection to the dogs that veterinary internists are seeing that have developed Cu-associated hepatitis.

Copper Content of Dog Foods 2017-2021

Data from state feed control samples of dog foods (including dry and wet diets for adult maintenance and all life stages) during the 5-year period from 2017 through 2021 indicate that Cu in dog foods tends to average between 20 to 30 mg Cu/kg DM with the maximum Cu concentration found in any one sample being 140 mg/kg DM (Figure 1). Figure 1 is a compilation of the Cu content of 1484 samples, roughly 300 per year, by diet type with the average Cu content assessed in all diets being fairly consistent over the 5 years (Figure 2).

Figure 1. Assayed Copper Content of Dog Foods 2017-2021 by Diet Type





Although the average (the X in the box) and median (the line in the box) Cu contents of dog foods do not appear to be increasing over the last 5 years, the data demonstrate there is a subset of foods with Cu content well above 1.5 times the interquartile range represented by the top whiskers of the plots and considered "outliers" (the individual dots). (See A Complete Guide to Box Plots | Tutorial by Chartio for a refresher on reading and interpreting Box and Whisker Plots.) **This indicates a need to establish for industry what good manufacturing and feeding practices would indicate the expected maximum Cu content to be in complete and balanced dog foods, and thus the need to indicate a maximum recommended quantity.** The upper whiskers in the plots would indicate a maximum of somewhere between 40 and 60 mg Cu/kg DM (see Figure 1). Although, again, a maximum of 60 mg Cu/kg DM may have no impact on the case prevalence for Cu-associated hepatitis in dogs.

European Union Environmental Maximum for Copper in Dog Food

The European Union (EU) has set a maximum Cu content for dog foods of 25 mg Cu/kg DM (6.25 mg Cu/1000 kcal of ME) based on environmental concerns.[23] The argument for adopting the EU's approach would be that dog food manufacturers marketing products internationally are already subjected to this maximum, as well as the US being subjected to similar environmental effects from excess Cu in diets. Twenty-five mg Cu/kg DM would be roughly twice the largest recommended AI for dogs set by the NRC. It would more tightly define what would be considered good manufacturing and feeding practices for the copper content of dog food and, with compliance by manufacturers, would decrease the copper content of roughly 50% of the dog foods currently on the market based on the 5-year sample results above. This is the most

severe restriction possible based on a science rationale and data. Whether it is as severe as is needed by dogs with genetic abnormalities for copper metabolism and clearance is unknown. However, a more severe restriction would not be science-based and would simply be an arbitrarily and capriciously imposed value.

Conclusion

Review of the scientific literature indicates that liver Cu concentrations and Cu-associated hepatitis in dogs has increased over the last 10-25 years. Although it is unknown if Cu amounts in dog foods have also increased during that time or if there is a direct causal relationship, data from the last 5 years demonstrates that dietary Cu typically exceeds adequate intake amounts. Thus, despite the lack of definitive data for a SUL of Cu in dog food, **it is this author's opinion that a maximum Cu concentration should be established to set a standard for good manufacturing and feeding practice** and to potentially protect the Cu-sensitive portion of the canine population that may be increasing in size.

The WG must decide what the Cu maximum should be to accomplish those goals. Based on the available science, the choices seemingly are:

- 25 mg Cu/kg DM, (6.25 mg Cu/1000 Kcal ME);
- 40-60 mg Cu/kg DM (10-15 Cu/1000 Kcal ME); or,
- 120 mg Cu/kg DM (30 mg Cu/1000 Kcal ME).

Whatever maximum the WG decides will likely be considered too great by Dr. Center and colleagues and too small by pet food manufacturers, but in the interest of protecting canine health, hopefully both sides will accept a compromise that provides their clients – the dog owners – increased confidence in the safety of dog foods.

Appendix 1

Table 1. Chronology of liver copper concentrations in dogs without hepatic injury[a]

Year of sampling	Hepatic Copper µg/g dry weight: mean ± SD, mean (range), median (range), upper limit clinically healthy dogs, or dogs without hepatitis, as reported.	Breeds	Number of liver samples	Ref.	Copper quantification method
1929	7	undeclared	undeclared	1	Colorimetric
1932	44	undeclared	undeclared	2	colorimetric *
1956	mean:80 (22-154)	undeclared	3	3	colorimetric**
1972	mean 52 (19-115) mean: 80 (22-154)	undeclared	21 & 3	4	colorimetric**
1981	mean: 304 ± 90 (150–500)	Beagles (40-197 day age)	20	5	Xray-spectroscopy
1982	mean: 200 ± 88 (91-377)	mixed bred dogs	31	6	AAS
1986	mean: 190 ± 56 (60-270)	13 mix bred dogs	13	7	AAS
1991	mean: 246 ± 48	Beagles (6 mth age)	4	8	AAS
1980-1995	median: 177 (93-453)	Labrador retriever	18	9	AAS or rhodanine digital scanning
1982-1988	median: 170 (104-310)	breeds not predisposed to copper hepatopathy	64	10	ICP-MS***
1982-1988	median: 249 (155-429)	breeds predisposed to copper hepatopathy	40	10	ICP-MS
1997-2013	median: 752 (101-3,810)	Labrador retriever	18	11	rhodanine digital scanning
2009-2015	median: 263 (166-399)	breeds not predisposed to copper hepatopathy	84	10	ICP-MS

*Elvehjem CA, Lindow CWJ. The determination of copper in biologic materials. Biol Chem 1929; 81:435-443.
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AAS = Atomic absorption spectroscopy
ICP-MS=Inductively Coupled Plasma – Mass Spectrometry.

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[a] Courtesy of Dr. Sharon A. Center

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Copper in Dog Foods Expert Panel
Final Report with Recommendations to the Pet Food Committee

Attachment 3

Copper Metabolism and its Implications for Canine Nutrition

Zinpro Corporation

Introduction

Copper nutriture of the dog has recently received increased attention in the United States and European Union due to reports of apparent copper-associated hepatitis (**CAH**). Recent trends in canine nutrition have led to new questions regarding proper dietary copper concentrations in canine diets. Recently, AAFCO and NRC guidelines for canine dietary copper concentrations have been questioned due to the lack of upper tolerable limits (**Center et al., 2021**). Given the reported increase in CAH and the current trends in canine nutrition, these concerns should be investigated. To identify the best course of action regarding these questions, it is important to consider the complexities of copper metabolism, available trace mineral research in dogs and other animal species, dietary ingredient composition and nutrient variability, and the potential effects of different supplemental sources of copper.

Historically, dog diets have been comprised of a mixture of grains, animal proteins, and byproducts. However, recent trends in consumer preference have shifted formulations towards grain-free diets containing higher concentrations of protein that has led to increased inclusion of novel ingredients including pulses, fresh meat, and organ meats, such as liver, in complete and balanced dog diets and treats. Additionally, raw meat and homemade formulations are gaining in popularity. Unsurprisingly, these dietary ingredients inherently provide different proportions of essential nutrients and, thus, result in different nutrient profiles for the animal. One of the essential nutrients that has recently come under greater scrutiny is copper due to its higher concentrations in these novel ingredients, as well as the inclusion of more bioavailable forms of copper in supplements and functional treats.

While there is limited scientific research in dogs to evaluate requirements and establish upper tolerable levels of most nutrients, it is imperative to review what research has been done in canine case studies and controlled studies in other species to identify what is applicable to dogs as well as in what areas questions and concerns remain. The following is a brief review of copper metabolism in monogastric animals and considerations that need to be understood when formulating dog diets to meet physiological requirements and assess the risk of potential toxicity. Focus should be placed on increasing our knowledge and understanding of copper metabolism by dogs before making changes in recommendations that could have broad implications for all dogs.

Copper Metabolism – Absorption, Distribution, Storage, and Excretion

Copper is an essential nutrient required by mammals due to its variety of roles in physiological processes necessary for basic function and health. However, given its chemical properties as a transition metal, concentrations need to be adequately balanced in diet formulations. Copper can be an acceptor or donator of electrons due to its two oxidation states, cuprous (Cu+) and cupric (Cu++) copper. Therefore, an excess of copper in cells could be detrimental due to potential free radical formation and subsequent oxidative stress (**Chen et al., 2020**). However, the capability of copper to switch readily between oxidation states is also responsible for its essential role in a variety of enzymatic and biochemical reactions. Notably, these include cytochrome c oxidase (electron transport chain function), lysyl oxidase (collagen and elastin formation), Cu/Zn superoxide dismutase (SOD; antioxidant defense), dopamine beta hydroxylase (neurotransmitter biosynthesis), tyrosinase (pigmentation), sulfhydryl oxidase (keratinization), and ceruloplasmin and hephaestin (iron homeostasis) (**Goff, 2017; Møller & Aaseth, 2022**).

Absorption

Circulating copper (Cu++) is sensed by intestinal enterocytes and converted by brush border reductase enzymes to Cu+, the form in which it can be absorbed by the enterocyte. The copper transporter 1 protein (**CTR1**) transports Cu+ across the apical membrane of the enterocyte. Small amounts of Cu+ can also be transported into the enterocyte via divalent metal transporter 1 (**DMT1**). Copper is immediately bound by chaperone proteins, glutathione (**GSH**) or metallothionein (**MT**), to reduce the risk of oxidative damage. The GSH-bound copper will subsequently be transferred to copper chaperone proteins that carry Cu+ to various cellular compartments or proteins responsible for export based on cellular and systemic copper needs (**Kaplan & Maryon, 2016; Goff, 2017; Chen et al., 2020).**

Cellular copper chaperones include copper chaperone for SOD (**CCS**) that carries Cu+ to SOD, a pivotal enzyme involved in antioxidant defenses; cytochrome c oxidase 17 (**COX17**) that carries Cu+ to the mitochondria for proper functioning of cytochrome c oxidase, an indispensable component of the electron transport chain and cellular energy metabolism; and antioxidant 1 (**ATOX1**) that carries Cu+ to ATPase copper transporting alpha (**ATP7A**) in the enterocyte or ATPase copper transporting beta (**ATP7B**) in the hepatocyte (**Goff, 2017; Chen et al., 2020**).

Once Cu+ is bound to ATP7A, it is transported through the cell via a transport vesicle to the basolateral membrane and subsequently released into circulation, bound to circulating proteins, mainly albumin, and transported to other tissues, mainly the liver. The liver is the main organ responsible for maintaining copper homeostasis. Hepatocyte reductase enzymes reduce circulating Cu++ to Cu+ and transport it into the cell via CTR1. Once in the hepatocyte, the same chaperone proteins present in the enterocyte will shuttle copper to the necessary cellular sites for enzyme function, protein synthesis, or excretion depending on cellular and systemic needs (**Goff, 2017**).

Storage and Distribution

The liver is responsible for synthesizing copper-containing proteins for transport to other tissues. This is facilitated via the hepatic ATPase, ATB7, present in the Golgi membrane, the protein factory of the cell. The major copper-containing protein produced in the liver is ceruloplasmin. Ceruloplasmin can carry copper to cells throughout the body. Additionally, ceruloplasmin is a ferroxidase enzyme. Ferroxidsae oxidizes ferrous (Fe++) to ferric (Fe+++) iron which is necessary for maintaining iron homeostasis. Roughly 40-70% of plasma copper is bound to ceruloplasmin (**Goff, 2017**).

Copper homeostasis is maintained via absorption efficiency, sequestration, storage, and excretion. When the animal has adequate copper stores, enterocyte CTR1 will be internalized and recycled for later use or degraded (**Chen et al., 2020**). At the same time, enterocyte MT production increases to sequester excess Cu+ and prevent enterocyte oxidative damage until it can transfer the Cu+ to ATOX to be incorporated into ATP7A for export to the liver for storage or excretion. Copper that remains bound to MT when an enterocyte dies and is shed will be excreted in the feces (**Goff, 2017**).

Excretion

The liver has a large capacity to store copper, but once that capacity has been met, ATP7B transports excess Cu+ out of the liver where it is excreted via bile. An animal's capacity for biliary excretion of copper is what determines its copper tolerance and is responsible for the vast differences in animal species' risk for copper toxicity. Even though liver copper storage capacity is high, there are events that increase risk for local and systemic oxidative damage due to Cu+ release. During physiological stressful events (ie. Inflammation, infection), liver protein turnover increases and has the potential to release the stored, highly pro-oxidant Cu+ locally and systemically, increasing the risk for widespread oxidative damage, ultimately causing cellular and tissue death.

Deficiency and Toxicity

Common signs of copper deficiency include loss of hair color, reduced fertility, impaired cellular immune response, and impaired connective tissue integrity. These deficiencies are not surprising given copper's role in multiple enzymes specific to these physiological functions and its indispensable role in cellular energy homeostasis. Immune function may be impacted by a copper deficiency before more common signs of deficiency are obvious. For example, a study in sheep suggested that Cu requirements increase during an immune challenge (**Suttle, 2012**). Additionally, ceruloplasmin, MT, and other copper-containing acute phase proteins increase during inflammation and infection that will result in increased binding of Cu and subsequent risk for secondary deficiencies due to the copper being unavailable to the animal for cellular and tissue homeostasis.

Common consequences of copper toxicity include decreased liver function, hemolysis, and cellular and tissue necrosis. Stress, inflammation, infection, or other immune challenges can result in increased liver protein turnover and increase Cu+ release into circulation where it can overwhelm carrier protein capacities and result in widespread cellular and tissue damage due to its strong pro-oxidant properties (**Goff, 2017**).

Both deficiency and toxicity signs can be delayed relative to the onset of innate or induced copper imbalances based on the nature of the perturbation. For example, deficiency signs may be delayed due to a lag between reduced copper concentrations and copper stores being depleted. At the same time, toxicities can be acute or chronic. Chronic copper toxicity can occur due to exposure to elevated dietary copper for long periods of time without signs of toxicity until the storage capacity of the liver is overwhelmed. Acute copper toxicity can occur after a physiologically stressful event (ie. inflammation, infection) where liver protein turnover is elevated and a high amount of Cu+ is released.

Considerations for Balanced Copper Nutrition in Dogs

Reports of copper-associated hepatitis (**CAH**) and inflammatory hepatic disease in dogs have increased over the last two decades. While risk of toxicity from copper is elevated compared to other micronutrients, it is vital to understand the various factors that affect copper metabolism to formulate canine diets that meet the animal's requirements while minimizing the risk for toxicity, given the important physiologic roles of copper mentioned above.

Genetic Predisposition to Copper Toxicities

There are two important factors to consider regarding canine CAH when determining recommendations for dietary copper inclusion; genetic predisposition and environmental influence (ie., diet).

Bedlington Terriers were the first breed to be recognized as having a causational mutation leading to CAH. A mutation in *COMMD1* led to impaired biliary excretion of copper, thus causing the liver to be overwhelmed by Cu+, leading to hepatic pathologies (**Dirksen & Fieten, 2017**). Labrador Retrievers were also recognized as having a genetic component of CAH risk, but it appeared to be much more complex than was the case for Bedlington Terriers. Only 12% of the heritability of CAH in Labrador Retrievers can be accounted for by genetic mutations identified to date and, therefore, environmental and/or other yet to be identified genetic factors are at play (**Dirksen & Fieten, 2017; Johnston et al., 2013; Strickland et al., 2018; Wu et al., 2020**). There are breeds other than Bedlington Terriers and Labrador Retrievers that have been identified as having suspected hereditary CAH and are, therefore, considered to be predisposed to copper toxicity and CAH. These include West Highland Terriers, Doberman Pinschers, and Dalmatians (**Spee et al., 2005; Dirksen & Fieten, 2017; Johnston et al., 2013; Strickland et al., 2018**).

In a recent (2018) retrospective study of 546 dogs, Strickland and colleagues reported evidence for significantly increased hepatic copper concentrations detected in dogs over the years of 1982-2015. This increase was not limited to dogs of breeds considered to be predisposed to CAH. The cutoff values for hepatic copper concentration used in this study were 300, 400, and 1000 ug Cu/g liver (dry weight basis) based on when hepatic injury was likely and individual clinic reference ranges. Importantly, the authors point out that normal hepatic copper concentrations for dogs are not clearly established and that definitive clinical relevance of the observed increases in hepatic copper concentrations remains elusive (**Strickland et al., 2018**). It is important to note that the dietary histories of these dogs were not available in this analysis.

Breeds that are predisposed to hereditary CAH may serve as good models to understand how diet may be used as a therapeutic agent to prevent and/or treat risk of serious liver pathologies. There is some evidence that feeding lower copper diets to Labrador Retrievers may decrease liver Cu concentrations (**Hoffmann et al., 2009; Fieten et al., 2012**). This area warrants further investigation.

Dietary Contributions to Copper Toxicity

It has been speculated that the increased incidence of CAH cases coincided with AAFCO recommendations to stop use of copper oxide as the source of supplemental Cu in dog diets due to its extremely low bioavailability (**Strickland et al., 2018; Center et al., 2021**). This speculation raises questions about the bioavailability of copper in various dog dietary ingredients and copper supplementation sources, as well as how those factors are affected by other nutrients and different physiological states. The current NRC recommendation for adult dogs at maintenance is 6 ppm Cu (total diet) based on a dietary energy concentration of 4000 kcal (DM basis; **NRC, 2006**) with higher concentrations recommended for puppies after weaning (11 pm, total diet) and females during late gestation and lactation (12.4 ppm, total diet). Minimum inclusion levels recommended by AAFCO are 7.3 ppm (total diet) for adult dogs at maintenance and 12.4 ppm (total diet) for growth and reproduction periods or for All Life Stages (DM basis; **AAFCO, 2019**). However, no upper tolerable limits have been established by either organization. It is important to distinguish that these recommendations are for minimum total copper concentrations, ie., dietary ingredient plus supplemental (premix) contributions, not minimum supplemental concentrations.

Copper bioavailability in ingredients used in dog diet formulations have, for the most part, not been studied in dogs and are generally unknown. However, extrapolations from other monogastric species have been made, mostly from chickens and pigs. These diets are mainly composed of corn and soybean meal and even though ingredient inclusion is somewhat consistent across the industry, there is still a large amount of variation and inconsistency in estimates of Cu bioavailability. In general, copper derived from common feed ingredients and additives in chicken and pig diets is considered to have a relative bioavailability of 50% compared to a copper sulfate standard, with a wide range of 10-50% being suggested in swine diets (**EFSA, 2016**).

As mentioned above, there is an increased prevalence of raw meat and grain-free/high proteinbased diets for dogs. Organ meats are inherently high in copper concentration and, therefore, the relative bioavailability of that copper needs to be considered and accounted for. Copper concentrations are higher in liver from ruminant species like beef and lamb but are much lower in chicken and turkey liver. There is also potential for variable bioavailability based on the form of liver used (ie., freeze dried or fresh) as well as the plane of nutrition for the animal that the liver was derived from. Aoyagi and colleagues (1993) utilized a chick bioassay to determine relative bioavailability of copper from freeze-dried liver of different animal origins. The following bioavailability values (%) relative to copper sulfate (100) were determined: 0 (pork), 21 (rat), 82 (beef), 83 (turkey), 113 (sheep), 116 (chicken – low Cu), 135 (chicken – high Cu). Additionally, they concluded that when fibrous ingredients (peanut hulls or soy mill run) were present, the bioavailability of copper sulfate was decreased by roughly 50% (**Aoyagi et al., 1993**). Although this study was conducted in the early 1990's, it still provides pivotal baseline information that can be used when formulating dog diets with increased inclusion of liver.

Taken together, copper bioavailability in common feed ingredients in monogastric livestock species is variable and inconsistent. As exemplified in Aoyagi's study, animal-derived high copper-containing feed ingredients, like liver, varies by species origin. There is limited research in dogs related to ingredient copper bioavailability and, therefore, further research is warranted to try to better estimate relative bioavailability that can be directly applied in canine nutrition.

Another important factor to consider when formulating dog diets is the interaction between nutrients and potential antagonisms that can occur. It is imperative that both dietary ingredients as well as supplemental contributions are taken into consideration when formulating complete diets. Additionally, copper contribution from treats and other daily supplements needs to be accounted for. Dietary copper sufficiency cannot be evaluated based on calculated copper concentration alone. There are important nutrient interactions that need to be accounted for. Zinc, iron, molybdate, and sulfur are all known to antagonize the amount of bioavailable copper. Zinc is a potent inducer of MT production that will preferentially bind and sequester Cu+ and thus increase the risk for a zinc-induced copper deficiency, regardless of dietary copper concentration. As mentioned above, homeostatic iron maintenance requires copper. However, excess dietary iron can also act as an antagonist to copper, thus complicating this nutrient interaction. A study in rats revealed that high dietary iron can cause copperdeficient anemia via disturbances in copper utilization after it has been absorbed (**Ha et al., 2017**). Additionally, Schultheiss and colleagues found that dogs with increased hepatic iron concentrations also had elevated liver copper concentrations (**Schultheiss et al., 2002**). While causation for which mineral may be driving the response cannot be determined, at the very least there appears to be a correlation between iron and copper hepatic accumulation. Basal ingredient iron contribution is elevated in raw meat and grain-free dog diets and is, therefore, an important consideration when determining copper supplementation in these formulations.

The concomitant increase of iron and copper concentrations was observed and compared among dogs with varying degrees of liver lesions (**Schultheiss et al., 2002**) and underlines the possibility of other mineral contributions to hepatic pathologies. Although there are little data about the interaction of copper and lead, Gori and colleagues (2021) observed increased liver lead concentrations in dogs with liver copper concentrations above 400 ppm (dry weight basis; **Gori et al., 2021**). Similar to the iron and copper relationship mentioned above, there is not sufficient evidence to determine causation versus correlation, but these results emphasize the need for further investigation of the overall nutrient status of dogs suffering from CAH.

Excess molybdate and sulfur can also be antagonistic to copper bioavailability. These nutrients can create complexes that bind copper and render it unavailable to the animal. While this may be of more concern in ruminant animals due to increased production of these complexes in the rumen, it is still worthy to note and be aware of when formulating monogastric animal diets, especially when determining sulfur-containing amino acid inclusion.

Assessing Copper Status

Formulating diets with adequate but not excess copper is made more complex by the lack of reliable, non-invasive copper status biomarkers. Currently, there are no copper status biomarkers for dogs; therefore, liver biopsy is necessary and considered the gold standard for copper toxicosis diagnosis (**Fieten et al., 2012**). However, there is evidence to support the potential variation of liver copper concentrations based on biopsy type and specimen size (**Johnston et al.,** 2009) and, therefore, caution should be taken when making comparisons across different studies. Current recommendation ranges used to determine potential copper toxicity are based on liver copper concentrations on a dry weight basis. Therefore, it is difficult to make comparisons and conclusions from studies based on liver copper concentrations on a wet weight basis (**Paβlack et al., 2014; Cedeño et al., 2016**).

Cedeño and colleagues (2020) recently reported that serum copper was increased in dogs with hepatic disease and inflammatory infections (**Cedeño et al., 2020**). However, serum copper concentrations do not correlate with hepatic copper concentrations in dogs (**Dirksen & Fieten, 2017**) and, therefore, caution should be taken when using this biomarker to evaluate risk for or diagnosis of liver pathologies.

While alanine aminotransferase (**ALT**) and alkaline phosphatase (**ALP**) are the most common biomarkers used to indicate hepatocellular injury, they are not usually altered until later stages of hepatopathy when liver damage has begun and can also be altered by multiple physiological disturbances. Given these factors, they are not effective at detecting subclinical copper imbalances and, therefore, may not allow detection before irreversible liver damage has occurred (**Dirksen & Fieten, 2017**).

Copper Sources

The most common sources of copper used in mineral premixes added to complete diets are copper sulfate or organic sources, such as amino acid chelated copper. For reference, the term organic is used herein and is interchangeable with chelated as is most commonly used in companion animal nutrition. Historically, copper oxide was also used but due to its extremely low availability to the animal (**Ledoux et al., 1991**), it is no longer recommended as a supplemental source of copper. The most common inorganic sources of Cu include sulfates and carbonates. When compared to bioavailability of reagent grade copper acetate (100%) in a chick bioassay, copper sulfate and carbonate were 88.5 and 54.3% bioavailable, respectively, while copper oxide was determined to be less than 1% bioavailable to the chick (**Ledoux et al., 1991**). A similar hierarchy of bioavailability from these inorganic Cu sources is observed in ruminant species (**Ledoux et al., 1995**).

The increased use of more bioavailable sources of copper supplements in combination with higher basal ingredient contributions of copper have been speculated as being one of the main driving forces for increased CAH cases (**Center et al., 2021**). However, emphasis should be placed on an effective and reliable supplemental source due to the high potential variability and inconsistency in diet ingredient copper contributions. This will allow nutritionists to include minimal supplemental concentrations to ensure the dog's requirements are met while also reducing the need to over-formulate copper in diets, thus reducing the risk of toxicity.

The currently available data suggest organic forms of supplemental Cu do not pose an increased risk of toxicity, as measured by liver copper concentrations. As mentioned previously, ruminant species tend to be at a higher risk for copper toxicity and, therefore, serve as a good model to evaluate potential issues among different forms of supplemental Cu. In a study comparing the effect of supplemental copper source and feeding regimen in sheep fed equivalent levels of copper as Cu sulfate or Cu-lysine complex, sheep feed Cu-lysine had significantly less final and overall change from initial liver copper concentrations, regardless of feeding regimen (**Luo et al., 1996**). Additionally, no difference in liver copper concentrations were detected among dairy cattle fed equivalent concentrations of Cu sulfate compared to Cu-lysine complex (**Chase et al., 2000**).

There are data in monogastric species to suggest no increased risk of toxicity due to organic supplemental forms of copper. Guo and colleagues (2001) evaluated different forms of organic copper (proteinates, lysine complex, amino acid chelate) in two different breeds of chickens. Overall, they observed slight increases in the bioavailability of copper from the organic sources compared to Cu sulfate (set at 100% bioavailable) as measured by liver copper concentrations, but there were no signs of toxicity reported (**Guo et al., 2001**). More importantly, the authors noted the different magnitude of response among the two different breeds that highlights the inherent limitations of species, breed, and experimental conditions when determining relative bioavailability (**Guo et al., 2001**). The growth-promoting characteristics of increased copper supplementation was compared among pigs fed two equivalent concentrations of Cu sulfate or

Cu-lysine complex. Like the other studies mentioned above, there was no observed increased risk of toxicity in pigs fed the organic Cu-lysine form. Averaged across 4 different experiments, pigs fed Cu-lysine had lower liver copper concentrations compared to pigs fed Cu sulfate, (**Coffey et al., 1994**).

Collaborative Next Steps for Maximum Canine Health and Longevity

Copper metabolism is a complex physiological process and homeostatic regulation is influenced by multiple factors including, but not limited to, variable copper bioavailability across and within ingredients, stage of development or reproduction, physiological stressors such as inflammation or immune challenges, and sufficient protocols for monitoring copper status. Canine nutrition is further complicated by a wide variety and quality of dietary ingredients and nutritional profiles of commercially available diets.

There is no current research evidence to suggest that the minimum total dietary copper recommendations provided by NRC and AAFCO are insufficient to meet basic physiological needs. However, this is assuming the above-mentioned factors that affect copper metabolism are not perturbed. The uncertainties around dietary ingredient copper contribution and the bioavailability of the copper present often leads to the common practice of formulating diets that all too often exceed those recommended levels due to the desire to provide a safety net for potential deficiency. However, this comes with a risk for nutrients such as copper that are more vulnerable to becoming toxic.

There are multiple opportunities for collaboration among veterinarians, nutritionists, and pet food manufacturers to meet the shared goal of providing pet parents dog food options they can be confident are supplying their dog the necessary nutrients to support an active and healthy life. Next steps to reach those goals might include:

1. Increased emphasis on creating a database that provides average concentrations of copper in common dog diet ingredients that would increase the industry's knowledge about variable basal ingredient copper contributions in complete diets.
2. Pet parent education about breeds at higher risk of copper toxicity.
3. Support for copper bioavailability research of individual dog diet ingredients as well as effects of ingredient combinations on bioavailability.
4. Utilization of reliable, consistent, high quality copper sources in mineral premixes at minimal inclusion concentrations (7 ppm Cu/kg diet).

Conclusions

There are many questions that remain regarding copper metabolism in dogs that will most likely continue to evolve as trends in canine nutrition come and go. Future research efforts should be placed on discovering reliable, non-invasive methods for evaluating copper status that are sensitive enough to detect imbalances prior to liver damage as well as increasing knowledge

about variation in ingredient copper bioavailability in dogs. Complex factors such as genetic predisposition and unpredictable physiological stressors need to be considered as well.

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