This Technical Report discusses 13 specific amino acids petitioned for addition to organic pet food. The scope of amino acids petitioned, which are listed in Table 1, is based on those defined as “required nutrients” by the National Research Council’s (NRC’s) Nutrient Requirements of Dogs and Cats (NRC, 2006). In this Technical Report, information is provided about the petitioned amino acids individually and collectively as appropriate and as allowed by available information. Two amino acids, taurine and methionine, are discussed as examples in some sections. These amino acids were the evaluated in recent Technical Reports (USDA, 2011a and 2011b) concerning petitioned uses in organic handling.

Table 1. Identity of Required Amino Acids in Pet Food

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>CAS Number</th>
<th>Trade Names</th>
<th>Other Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>(S)-2-Amino-5-guanidinopentanoic acid</td>
<td>74-79-3</td>
<td>Arginine (L-)</td>
<td>EINECS: 230-571-3</td>
</tr>
<tr>
<td>Methionine</td>
<td>2-amino-4-((methylthio)butanoic acid</td>
<td>63-68-3 (L-); 59-51-8 (DL-)</td>
<td>Mepron®; Alimet®</td>
<td>EINECS: 200-432-1</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2-amino-3-sulfanylpropanoic acid</td>
<td>52-90-4; 3374-22-9 (DL-)</td>
<td>L-Cysteine; L-Cysteine Hydrochloride Monohydrate</td>
<td>EINECS: 222-160-2</td>
</tr>
<tr>
<td>Lysine</td>
<td>2,6-diaminohexanoic acid</td>
<td>56-87-1 (L-); 70-54-2 (DL-)</td>
<td>VitaLys®; L-Lysine Premium®</td>
<td>EINECS: 200-740-6</td>
</tr>
<tr>
<td>Taurine</td>
<td>2-aminoethane sulfonic acid</td>
<td>107-35-7</td>
<td>Taurine; AI3-18307; O-Due; Taurina; Taurak</td>
<td>EINECS: 203-483-8</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>(2S)-2-amino-3-(IH-indol-3-yl)propanoic acid</td>
<td>73-22-3 (L-); 54-12-6 (DL-)</td>
<td>TryptoPure®; L-Tryptophan</td>
<td>EINECS: 200-194-9</td>
</tr>
<tr>
<td>Threonine</td>
<td>2-Amino-3-hydroxybutanoic acid</td>
<td>72-19-5 (L-); 80-68-2 (DL-)</td>
<td>L-Threonine; DL-Threonine;</td>
<td>EINECS: 201-300-6</td>
</tr>
<tr>
<td>Histidine</td>
<td>2-Amino-3-((H-imidazol-4-yl)propanoic acid</td>
<td>71-00-1 (L-); 4998-57-6 (DL-)</td>
<td>L-Histidine</td>
<td>EINECS: 225-660-9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2-Amino-3-methylpentanoic acid</td>
<td>73-32-5 (L-); 328-39-2 (DL-)</td>
<td>L-Isoleucine</td>
<td>EINECS: 207-139-8</td>
</tr>
<tr>
<td>Valine</td>
<td>2-Amino-3-phenylpropanoic acid</td>
<td>72-18-4 (L-); 516-06-3 (DL-)</td>
<td>L-Valine</td>
<td>EINECS: 208-220-0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2-Amino-3-phenylpropanoic acid</td>
<td>63-91-2 (L-); 150-30-1 (DL-)</td>
<td>L-Phenylalanine</td>
<td>EINECS: 205-756-7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>L-2-Amino-3-(4-hydroxyphenyl)propanoic acid</td>
<td>60-18-4 (L-); 556-03-6 (DL-)</td>
<td>L-Tyrosine</td>
<td>EINECS: 209-113-1</td>
</tr>
</tbody>
</table>
Summary of Petitioned Use

The petitioner, the Pet Food Institute, requests the addition of synthetic amino acids (i.e., arginine, methionine, cysteine, lysine, taurine, tryptophan, threonine, histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine) categorized as required in pet food to the National List for use in fortifying commercial pet foods labeled as organic. Specifically, the petitioner, the Pet Food Institute, is seeking the addition of the amino acids listed in Table 1 to the National List at 7 CFR 205.603, Synthetic Substances Allowed for Use in Organic Livestock Production. Petitioned amino acids would be used in the following pet food products (Pet Food Institute, 2012):

- Wet cat food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction;
- Semi-moist cat food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction;
- Dry cat food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction;
- Wet dog food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction;
- Semi-moist dog food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction;
- Dry dog food formulations labeled as complete and balanced for adult maintenance, and for growth and reproduction; and
- Pet treats.

Characterization of Petitioned Substance

Composition of the Substance:

The general structure of an amino acid is shown below in Figure 1. All amino acids have an amino group or amine (NH\(_2\)) adjacent to a carboxyl (COOH) group on a carbon (Osuri, 2003). The approximately 23 standard amino acids differ according to the varied structures in their side chains (or R groups). Three amino acids (leucine, isoleucine, and valine) are classified as a “branched-chain amino acid.” These amino acids have branching aliphatic side-chains (in which a carbon atom is bound to more than two other carbon atoms).

![Figure 1. General structure of an amino acid](source)

In Figures 2 and 3, the structure of taurine and methionine are provided as examples of the specific petitioned amino acids. These two amino acids contain sulfur, an element not found in the structure of most amino acids. Taurine and methionine are commonly added to fortify pet foods and serve as examples of required amino acids. Taurine is not considered a true amino acid because it does not contain a carboxyl group and is not incorporated into proteins (Schuller-Levis et al., 2003).

![Figure 2. Structure of Taurine (NH\(_2\)CH\(_2\)CH\(_2\)SO\(_3\)H, or C\(_2\)H\(_7\)NO\(_3\)S)](source)
Properties of the Substance:

Both the amino (H₂N) and carboxylic acid (COOH) groups of amino acids ionize readily. The carboxyl acid groups are in their conjugate base (carboxylate) form and an amino acid may act as an acid and also a base. Amino acids are considered dipolar ions because they can possess charged groups with opposite polarity. The ionic property of the amino acid side chains influences the physical and chemical properties of free amino acids and amino acids in proteins (Osuri, 2003). As a class of substances, amino acids have a relatively low vapor pressure (U.S. Department of Commerce National Standards Board, 1966).

Taurine is generally found as a white crystalline powder or solid. It has a pH of 5 in a 5% aqueous solution and a pH between 4.5 and 6 at 62.6 grams (g)/liter (L) at 25°C as a solid. Taurine has a melting point of >300°C (>572°F). Taurine is completely soluble in water (solubility of 65 g/L at 12°C) (Sigma Aldrich, 2010; Fischer Scientific, 2008).

Methionine is typically found as a white solid or white crystalline powder. Methionine is asymmetric, forming both an L- and a D-enantiomer. An enantiomer is is one of two stereoisomers that are mirror images of each other that are non-superposable (not identical), meaning that they are similar with the exception of orientation. Methionine hydroxy analog (MHA) is available in liquid form. It is soluble in water, methanol, alkali solutions, and mineral acids, and is slightly soluble in ether. Methionine is stable under normal temperature and pressure, but is incompatible with strong oxidizing agents (Acros, 2009). Hazardous decomposition products of methionine include nitrogen oxides, carbon monoxide, oxides of sulfur, and carbon dioxide (Pestell Minerals and Ingredients, 2008).

Specific Uses of the Substance:

Twenty amino acids are present in animal proteins, and other less common amino acids exist naturally and have biological functions. A growing number of non-protein amino acids that do not occur in nature have been synthesized. There are two classifications for amino acids of dietary protein; essential - those that the body cannot manufacture in sufficient quantities and non-essential - those that the body can manufacture in sufficient quantities (Cusick, 1997). All essential amino acids are defined as “required nutrients” by the National Research Council’s (NRC’s) Nutrient Requirements of Dogs and Cats in addition to taurine, cysteine, and tyrosine (Pet Food Institute, 2012; National Research Council, 2006). Amino acids considered “required” by NRC are petitioned for use in organic pet food products.

Amino acids have a variety of uses including nutrition supplements, fertilizers, and inputs to food technology and other industrial processes. Examples of the industrial applications of amino acids include the production of biodegradable plastics, drugs, and chiral catalysts. In crop production, amino acids act as chelating/complexing agents for cation nutrients, plant growth regulators, substrate for microbiological products, and a fertilizer source of nitrogen (USDA, 2007).

The pharmaceutical industry uses a variety of amino acids for making intravenous nutrient solutions for pre- and post-operative care (HolisticMed, undated). Amino acids are also commonly used as human dietary supplements and in the manufacture of artificial sweeteners (aspartame). Taurine has been added to many infant formulas since the late 1980s and is now included in most organic brands (USDA, 2011a). Taurine is also added in high concentrations to energy drinks such as Red Bull (1000 mg), Monster (2000 mg), and others.
Amino acids are frequently used as a feed additive in conventional poultry and swine production (FAO, undated). For optimum health and performance, an animal’s diet should contain adequate quantities of all nutrients needed, including amino acids. A shortage in the diet of one or more of the essential amino acids could restrict animal growth, reduce feed efficiency, and may cause nutritional deficiency. Supplementation with isolated amino acids increases feed conversion efficiency, thus lowering feed costs per unit of weight gain or production (FAO, undated). Methionine is considered to be the first limiting amino acid in corn-soy poultry diets because if the diet is deficient in methionine the usefulness of other amino acids is limited, even if those other amino acids are present in sufficient quantities. The literature indicates that cysteine (specifically total cysteine + methionine, which are both sulfur amino acids) and lysine are the next limiting amino acids (NRC, 1994; Cheeke, 1999; Gehrke et al., 1987). Lysine is also a major component of broiler and pig feed and it is estimated that the use of 1 ton of lysine can save the usage of 33 tons of soybean meal (FAO, undated). In swine production, lysine is considered the limiting amino acid for growing pigs (Rothlisberger, 2005). Taurine may also be added to conventional chicken feed, although its benefits to laying hens, poultry broilers, and turkeys are questionable (USDA, 2011a).

Amino acids are used in conventional livestock healthcare to create a variety of conditions. Methionine is specifically used as a urine acidifier because excretion of its sulfate anion lowers urine pH. Methionine may assist in dissolving and/or preventing uroliths, kidney stones, bladder stones, or urologic syndromes thought to be caused by struvite crystals or uroliths (Lewis et al., 1987). Methionine, important in mobilizing fat and transporting fat from cells, is sometimes used to assist in the treatment and/or prevention of hepatic lipidosis, or fatty liver disease in livestock (USDA, 2001). However, it appears there are insufficient data to support its efficacy in treating this condition (Merck Veterinary Manual, 2011).

Amino acids are also critical to the diets of dogs and cats and protein is an important component of both cat and dog food (Cusick, 1997). Natural sources of amino acids, the true “building blocks” of protein, are discussed in Evaluation Question #11. Amino acids are common ingredients added to some commercial pet food products. The bodies of dogs and cats can manufacture 13 of the 23 known amino acids. The other 10 essential amino acids must come from dietary meat and plant sources (Cusick, 1997). In addition, taurine is a common ingredient added to some commercial dog and virtually all cat food because it is considered essential for cats. Taurine is considered an essential dietary nutrient for cats but dispensable for dogs fed adequate quantities of sulfur-containing amino acids (USDA, 2011a). Please see “Action of the Substance” for additional discussion on why taurine is considered essential for cats only.

**Approved Legal Uses of the Substance:**

**Animal Feed and Pet Food**

The Association of American Feed Control Officials (AAFCO), a voluntary membership association of local, state, and federal agencies required by law to regulate the sale and distribution of animal feed and medications, is considered the authority on pet nutrition in the United States. While AAFCO has no regulatory power, it has established a uniform code that has become the standard on which states base their feed laws and regulations (AAFCO, undated). As a result, pet food makers must follow this standard as well as regulations set forth by the FDA. In order for pet foods to be labeled “complete and balanced” by AAFCO, they must meet the nutrition standards of the AAFCO Dog or Cat Food Nutrient Profile. These nutrient profiles are provided below (FDA, 1997).

Amino acids do not appear on the National List for use in livestock feed. However, USDA requires that certain standards be met for livestock health care practices. Specifically, livestock must be provided with a feed ration sufficient to meet nutritional requirements, including vitamins, minerals, protein and/or amino acids, fatty acids, energy sources, and fiber (ruminants) (7 CFR 205.238(1)). The National Organic Program (NOP) Final Rule defines “livestock” to include cattle, sheep, goats, swine, poultry, or equine animals (7 CFR 205.2); pets, including dogs and cats, are not included in this definition.
Table 2. AAFCO Dog Food Nutrient Profiles

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Units DM Basis</th>
<th>Growth and Reproduction Minimum</th>
<th>Adult Maintenance Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>%</td>
<td>22.0</td>
<td>18.0</td>
<td>NA</td>
</tr>
<tr>
<td>Arginine</td>
<td>%</td>
<td>0.62</td>
<td>0.51</td>
<td>NA</td>
</tr>
<tr>
<td>Histidine</td>
<td>%</td>
<td>0.22</td>
<td>0.18</td>
<td>NA</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>%</td>
<td>0.45</td>
<td>0.37</td>
<td>NA</td>
</tr>
<tr>
<td>Leucine</td>
<td>%</td>
<td>0.72</td>
<td>0.59</td>
<td>NA</td>
</tr>
<tr>
<td>Lysine</td>
<td>%</td>
<td>0.77</td>
<td>0.63</td>
<td>NA</td>
</tr>
<tr>
<td>Methionine-cystine</td>
<td>%</td>
<td>0.53</td>
<td>0.43</td>
<td>NA</td>
</tr>
<tr>
<td>Phenylalanine-tyrosine</td>
<td>%</td>
<td>0.89</td>
<td>0.73</td>
<td>NA</td>
</tr>
<tr>
<td>Threonine</td>
<td>%</td>
<td>0.58</td>
<td>0.48</td>
<td>NA</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>%</td>
<td>0.20</td>
<td>0.16</td>
<td>NA</td>
</tr>
<tr>
<td>Valine</td>
<td>%</td>
<td>0.48</td>
<td>0.39</td>
<td>NA</td>
</tr>
<tr>
<td>Taurine</td>
<td>%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 3. AAFCO Cat Food Nutrient Profiles

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Units DM Basis</th>
<th>Growth and Reproduction Minimum</th>
<th>Adult Maintenance Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>%</td>
<td>30.0</td>
<td>26.0</td>
<td>NA</td>
</tr>
<tr>
<td>Arginine</td>
<td>%</td>
<td>1.25</td>
<td>1.04</td>
<td>NA</td>
</tr>
<tr>
<td>Histidine</td>
<td>%</td>
<td>0.31</td>
<td>0.31</td>
<td>NA</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>%</td>
<td>0.52</td>
<td>0.52</td>
<td>NA</td>
</tr>
<tr>
<td>Leucine</td>
<td>%</td>
<td>1.25</td>
<td>1.25</td>
<td>NA</td>
</tr>
<tr>
<td>Lysine</td>
<td>%</td>
<td>1.20</td>
<td>0.83</td>
<td>NA</td>
</tr>
<tr>
<td>Methionine-cystine</td>
<td>%</td>
<td>1.10</td>
<td>1.10</td>
<td>NA</td>
</tr>
<tr>
<td>Methionine</td>
<td>%</td>
<td>0.62</td>
<td>0.62</td>
<td>1.50</td>
</tr>
<tr>
<td>Phenylalanine-tyrosine</td>
<td>%</td>
<td>0.88</td>
<td>0.88</td>
<td>NA</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>%</td>
<td>0.42</td>
<td>0.42</td>
<td>NA</td>
</tr>
<tr>
<td>Threonine</td>
<td>%</td>
<td>0.73</td>
<td>0.73</td>
<td>NA</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>%</td>
<td>0.25</td>
<td>0.16</td>
<td>NA</td>
</tr>
<tr>
<td>Valine</td>
<td>%</td>
<td>0.62</td>
<td>0.62</td>
<td>NA</td>
</tr>
<tr>
<td>Taurine (extruded)</td>
<td>%</td>
<td>0.10</td>
<td>0.10</td>
<td>NA</td>
</tr>
<tr>
<td>Taurine (canned)</td>
<td>%</td>
<td>0.20</td>
<td>0.20</td>
<td>NA</td>
</tr>
</tbody>
</table>

Synthetic methionine is currently included on the National List (7 CFR 205.603(d)) for use in organic livestock production as a feed additive. Specifically, DL-methionine, DL-methionine-hydroxy analog, and DL-methionine-hydroxy analog calcium can be used in organic poultry production until October 1, 2012, at the following maximum levels of synthetic methionine per ton of feed: laying chickens–4 pounds; broiler chickens–5 pounds; and turkeys and all other poultry–6 pounds. The NOP has published a proposed rule that indicates that after October 1, 2012, the allowed levels will be reduced to 2 pounds for laying chickens, 2 pounds for broiler chickens, and 3 pounds for turkeys and other poultry through October 1, 2015 (76 CFR 13501; see OFPA, USDA Final Rule” section).

Specific amino acids are approved by the United States Food and Drug Administration (FDA) for use as a nutritional supplement in animal feeds. In addition, taurine is approved by the FDA for use as a nutritional supplement.
supplement in conventional chicken feed at concentrations of $<0.054\%$ (21 CFR 573.980). FDA regulates pet food in a similar way to livestock animal feed. The Federal Food, Drug, and Cosmetic Act (FFDCA) stipulates that all animal foods “be safe to eat, produced under sanitary conditions, contain no harmful substances, and be truthfully labeled” (FDA, 2011). Canned food is further required to conform to low acid regulations to ensure the food does not contain microorganisms that could make pets ill. Foods do not need to be approved by the FDA before they go on the market; however, additives including minerals, vitamins or other nutrients, flavorings, preservatives, or processing aids must be generally recognized as safe (GRAS) for their intended use (21 CFR 582 and 584) or have approval as food additives (21 CFR 570, 571 and 573).

**Human Food Additive and Dietary Supplement**

Amino acids do not appear on the National List for use in organic handling of food for human consumption. The following amino acids have been recently petitioned to the National List for use in organic infant formula: L-carnitine, L-methionine, and taurine.

The FDA does not regulate human dietary supplements in the same way as drugs or animal feed additives; generally, manufacturers do not need to register their products with FDA or get approval before producing and selling supplements for human consumption. The FDA is responsible for taking action regarding an unsafe product after it reaches the market and to make sure the supplement’s label is accurate and not misleading (FDA, 2005). However, the following required amino acids are considered by FDA to be Generally Recognized as Safe (GRAS):

- Arginine 21 CFR 582.5145
- Methionine 21 CFR 582.5475
- Cysteine 21 CFR 582.5271
- Lysine 21 CFR 582.5411
- Taurine \(^1\) 21 CFR 573.980
- Tryptophan 21 CFR 582.5915
- Threonine 21 CFR 582.5881
- Histidine 21 CFR 582.5361
- Isoleucine 21 CFR 582.5381
- Leucine 21 CFR 582.5406
- Phenylalanine 21 CFR 582.5590
- Tyrosine 21 CFR 582.5920
- Valine 21 CFR 582.5925

With the exception of methionine and cysteine, all amino acids referenced in 21 CFR 582 are declared GRAS for both the L- and DL- form. Cysteine is declared GRAS for L- form only, and for methionine no reference is made to the L- or DL form (21 CFR 582.5475).

**Action of the Substance:**

The essential amino acids and some of their functions in pets are as follows:

- **Arginine:** stimulates immune system response by enhancing T-cell production, has a protective effect of toxicity of hydrocarbons and intravenous diuretics, is related to the elevated ammonia levels and cirrhosis of the liver by detoxifying ammonia, and induces release of growth hormone from the pituitary gland;
- **Methionine:** assists gall bladder functions by participating in the synthesis of blue salts, helps to prevent deposits and cohesion of fats in the liver due to lipotropic function, is related to the

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\(^1\) Taurine does not appear on the list of GRAS food additives (21 CFR 582), but as discussed above, it is allowed as a nutritional supplement in chicken feed (21 CFR 573.980; FDA, 2011) and thus considered an approved food additive in conventional products.
synthesis of choline, balances the urinary tract pH, and gives rise to taurine (an important neuroregulator in the brain);  
- **Cysteine**: major constituent of hair and glutathione;  
- **Lysine**: promotes bone growth in puppies, stimulates secretion of gastric juices, and is found in abundance within collagen and muscle and connective tissues;  
- **Taurine**: involved in growth and fetal development, reproduction, neuro-modulation, sight, hearing, heart function, osmoregulation, fat emulsification, neutrophil function, immune response, antioxidation, and bile acid and xenobiotic conjugation and acts as an anticonvulsant;  
- **Tryptophan**: produces serotonin that induces sleep, is a precursor for the vitamin niacin in treating and preventing pellagra, and is a vasoconstrictor that appears to aid in blood clotting mechanisms; studies indicate a lack of tryptophan and methionine together can cause hair loss;  
- **Threonine**: regulates energy draw requirements, works with phenylalanine in mood elevation or depression and skin pigmentation, manufactures adrenalin, and precurses thyroid hormone;  
- **Histidine**: widens small blood vessels thereby aiding early digestion by stimulating stomach acid secretion; releases histamines; is associated with pain control and arthritis;  
- **Isoleucine and Leucine**: see Valine;  
- **Phenylalanine**: stimulates enzymes related to appetite control, increases blood pressure in hypotension, works with minerals in skin and hair pigmentation, gives rise to tyrosine, and produces adrenalin and noreadrenalin;  
- **Valine**: these three amino acids work together and regulate protein turnover and energy metabolism; are stored in muscle tissue and are released to be converted into energy during times of fasting or between meals; and  
- **Tyrosine**: made in animals only from phenylalanine; keratin protein composition of the hair.  
Sources: Cusick, 1997; NRC, 2006

Traditionally, dogs have made their own taurine from other amino acids, however some heart conditions typically observed in cats deficient in taurine are now also being observed in older dogs. Changes in the content of commercially produced dry dog foods (i.e., high content of cereal-grains and byproducts rather than true meat/poultry/fish) may have resulted in a potential need to add taurine to dog foods for older, larger canine breeds (Ko et al., 2007).

In cats, taurine is considered an essential amino acid because cats are not able to synthesize it on their own. A number of studies have described immunological abnormalities leading to decreased immune system function in cats fed taurine-free diets (Schuller-Levis et al., 2003). Other studies indicate that cats with diets not supplemented with taurine have more miscarriages, fewer live births, and a lower kitten survival rate than cats with adequately supplemented diets (Sturman and Messing, 1991). Because of these studies, most cat food is supplemented with taurine (VCA Animal Hospitals, undated). While not necessarily essential for all dogs, taurine supplementation may be beneficial for certain dog breeds. A recent study in Newfoundland dogs found a high incidence of low plasma taurine in the population. The dilated cardiomyopathy (a common condition in this breed) found in some of the study dogs was reversed after taurine supplementation (Backus et al., 2006).

**Combinations of the Substance:**

Amino acids are combined in feed rations of grains, beans, oilseeds, and other meals with antioxidants, vitamins, and minerals (Pond et al., 1995). In conventional agricultural feed products, amino acids also are combined with antibiotics and hormones, which are not permitted to be fed to organic livestock.

In pet foods, protein is derived from both plant and animal sources. Most protein ingredients contain insufficient amounts of one or more amino acids and are thus inefficient if used as the only source for supplying dietary protein. Inefficiencies can be eliminated by combining various protein sources in order to complement the deficiencies of one source with contents of another. For example, soybean meal and corn act as complements because the amino acids which are deficient in one are present in the other (Purina, 2012).
Amino acids are combined in pet food with sources of carbohydrate, which may make up 40 to 55% of dry diets in dog food and is found in lesser amounts in typical cat foods. Grains and/or flours from corn, oats, rice, barley, wheat, or sorghum are a primary source of carbohydrates (Purina, 2012).

Pet foods typically contain a number of added vitamin supplements (e.g., vitamins A, B12, D3, and niacin), trace minerals and elements (e.g., iron and manganese). Amino acids have not been identified as a component of or precursor to any other substance on the National List for pet food uses.

**Status**

**Historic Use:**

Cysteine and leucine were discovered in the mid-1800s. In the late 1800s, arginine and histidine were identified; however the relationship between amino acids and protein molecules was not yet understood. In 1899, Emil Fischer synthesized and identified additional amino acids, but more importantly illustrated how amino acids combined with each other inside the protein molecule. However, the nutritional significance of amino acids were not discovered until 1901 when Frederick Gowland Hopkins identified tryptophan and concluded that if proteins were not nutritionally identical then the amino acids they contained determined whether or not life is sustained. In the early 1900s, the experimental works of Osborne and Mendel supported this conclusion and illustrated that many amino acids (particularly lysine and tryptophan) came only from dietary sources and are not synthesized in the body. In 1935, threonine was isolated and it was determined that specific amino acids could be synthesized by humans and mammals and other amino acids required dietary supplementation (Lerner and Lerner, 2002).

Modern pet food was first introduced in the late 1800s when Spratt’s dog cakes, a combination of wheat, beet root, vegetables and beef blood, were created. This early form of pet food was not considered nutritionally balanced. The use of bagged and canned foods became popular in the United States after World War II (Olson, 2010). Taurine was first recognized as a necessary component of the domestic cat’s diet in the mid-1970s to early 1980s, resulting in taurine supplementation of cat food (VCA Animal Hospitals, undated).

**OFPA, USDA Final Rule:**

Amino acids (as a category) do not currently appear on the National List and are not currently listed for use in pet foods labeled as organic. Amino acids are included as a nutritional requirement for livestock feed ration under livestock health care practices (7 CFR 205.238(a)(2)).

In 2008, the National Organic Standards Board (NOSB) adopted a recommendation for organic pet food. This recommendation set forth that pet food regulations are a better fit under the livestock provisions of the NOP Final Rule and pointed out that, historically, pet food is regulated by states as a subset of livestock. Ingredients and additives permitted in pet food are regulated similarly to livestock feed. However, pets including dogs and cats are not included in the current regulatory definition of “livestock.” The NOSB proposed the addition of several regulatory definitions, including “pet,” “pet food,” etc. The NOSB clarified that the definition of livestock is to specifically not include pets or specialty pets (NOSB, 2008).

NOSB recommended that the product composition requirements for organic pet food be similar to those for livestock, but that labeling categories be the same as for processed human food. Further, this recommendation proposes that eligible label claims for organic pet food match the requirements for human food: i.e., a minimum of 70% organic ingredients for a “made with organic” claim, and at least 95% organic content for an “organic” claim (NOSB, 2008). The petitioner has requested that amino acids be included on the National List as a categorical addition because the absence of any single required amino acid prohibits a pet food product from being labeled as complete and balanced. The NOSB’s recommendation proposed the following materials for possible petition to the National List (205.603(d) Feed additives) for use in pet food (NOSB, 2008):
• L-arginine - for pet food (amino acid)
• D-L Methionine for pet food (amino acid)
• Carnitine\(^2\) for pet food (amino acid)
• L-cysteine for pet food (amino acid)
• L-lysine, L-lysine monochloride for pet food (amino acids)
• Taurine for pet food (amino acid)
• L-tryptophan for pet food (amino acid)

General uncertainty over the nutritional status of amino acids used in organic agriculture has focused on the issue that amino acids are neither a vitamin nor a mineral. In 1995, the NOSB wrote “The Use of Nutrient Supplementation in Organic Foods” for the Secretary of Agriculture, which stated:

Upon implementation of the National Organic Program, the use of synthetic vitamins, minerals, and/or accessory nutrients in products labeled as organic must be limited to that which is required by regulation or recommended for enrichment and fortification by independent professional associations (USDA, 2011a).

The NOSB clarified that the term “accessory nutrients” meant “nutrients not specifically classified as a vitamin or a mineral but found to promote optimum health.” However, confusion arose after the National List was established because an additional annotation (National List §205.605(b)) stated, “Nutrient Vitamins and Minerals, in accordance with 21 CFR 104.20, Nutritional Quality Guidelines for Foods, would be allowed for organic agriculture (USDA, 2011a).” Originally, the NOP interpreted that under 21 CFR 104.20(f), which states, “Nutrient(s) may be added to foods as permitted or required by applicable regulations established elsewhere in this chapter,” amino acids and other nutrients not specifically listed in the regulation were permissible. However, after further discussion with the FDA, a memorandum (USDA, 2010) from NOP to the NOSB clarified that 21 CFR 104.20(f) pertained only to substances listed in 21 CFR 103.20(d), which does not include amino acids.

The NOP advised certifying agencies that pet food could be certified based on standards for human organic food. Although synthetic vitamins and minerals are FDA approved for use in pet food, the NOP later clarified that since the NOP National List refers to human food fortification and not pet food, new guidance would be developed to provide for the organic certification of complete and balanced pet food (USDA, 2010). The NOP is in process of drafting proposed regulations for organic pet food.

International:

According to the Canadian General Standards Board, organic operators may not use “feed and feed additives, including amino acids and feed supplements that contain substances not in accordance with CAN/CGSB-32.311, Organic Production Systems - Permitted Substances Lists (CAN/CGSB-32.310-2006).” However, on the Permitted Substances List, nonsynthetic amino acids are permitted and exceptions are made for synthetic DL-methionine, DL-methionine hydroxy analog, and DL-methionine hydroxy analog calcium until October 1, 2010. No further amendments to this exception were identified.


The livestock nutrition section of the standards set forth by the Codex Alimentarius Commission (2010) states that for additives and processing aids, “antibiotics, coccidiostatics, medicinal substances, growth promoters or any other substance intended to stimulate growth or production shall not be used in animal feeding” (Codex Alimentarius Commission, 2010).

Furthermore, for feedstuffs and nutritional elements, the guidelines specify the following criteria:

\(^2\) Carnitine is not included in the petition submitted to the NOP by the Pet Food Institute (2012).
(1) Feedstuffs of mineral origin, trace elements, vitamins, or provitamins can only be used if they are of natural origin. In case of shortage of these substances, or in exceptional circumstances, chemically well-defined analogic substances may be used.

(2) Synthetic nitrogen or nonprotein nitrogen compounds shall not be used.

The second point appears to also prohibit synthetic amino acids. Nonprotein nitrogen compounds include substances such as urea and ammoniated materials (AAFCO, 2001). In the technical literature, nonprotein nitrogen is considered to include “free amino acids, amino acid amides, glucosides containing nitrogen, nucleotides, urea, nitrates, ammonium salts and other low-molecular weight compounds containing nitrogen” (Boda, 1990).

The International Federation of Organic Agriculture Movements (IFOAM) prohibits the use of “amino-acid isolates” in their norms (IFOAM, 2010).

The Japan Ministry of Agriculture, Forestry, and Fisheries do not mention the allowed or prohibited status of amino acids in organic livestock feed. However, the standard states the following as permitted (JMAFF, 2005):

Feed additives (except for those produced by using antibiotic and recombinant DNA technology), which are natural substances or those derived from natural substances without being chemically treated. In case of a difficulty to obtain feed additives listed in 8, the use of similar agents to the described food additives are permitted only for supplementing nutrition and effective components in feeds.

This suggests that amino acids may be allowed if natural substitutes are not available.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: What category in OFPA does this substance fall under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

(A) Amino acids petitioned for use in pet foods are considered nutrients. Methionine, cysteine, and taurine are sulfur-containing substances. The other required amino acids do not contain sulfur.

(B) Amino acids from synthetic sources are petitioned specifically. When used as a feed additive for livestock and/or companion animals, arginine, methionine, cysteine, lysine, taurine, tryptophan, threonine, histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine are not considered to be inert ingredients, as defined under 7 CFR 205.2 because they are not generally included in EPA-regulated pesticide products.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

The three general approaches most widely used to manufacture synthetic amino acids include direct chemical synthesis, fermentation, and bioconversion using enzymes. Choosing between manufacturing processes typically depends on available technology, costs of raw material, market prices and sizes, cost of...
running fermentation versus synthesis reactions, and the environmental impact of the process itself (Ikeda, 2003).

Chemical synthesis:

A large number of chemical reactions can be used to synthesize amino acids. An advantage of chemical synthesis is that it can be carried out on a very large scale for commercial application and often in a continuous manner. However, the synthesis reactions generally produce racemic mixtures (i.e., containing equal amounts of left- and right-handed enantiomers of a chiral molecule) of the amino acid. Therefore, it may be necessary to isolate the desired enantiomer recycle the undesired one (Ault, 2004). For example, the process for the isolation of cysteine produces racemic mixtures of enantiomers following the reaction of ammonia, hydrogen cyanide and mercaptaldehyde. L-cysteine monohydrochloride can also be produced by an enzymatic process (USDA, 2007).

Though methionine can be produced in the laboratory using fermentation methods, it is generally produced via chemical synthesis using the following reaction methods:

- The reaction of acrolein with methyl mercaptan in the presence of a catalyst (Fong et al., 1981).
- The reaction of propylene, hydrogen sulfide, methane, and ammonia to make the intermediates acrolein, methylthiol, and hydrocyanic acid (DeGussa, 1995, 1996);
- Use of the Strecker synthesis method with α-methylthiopropionaldehyde as the aldehyde (Fong et al., 1981); and
- The reaction of 3-methylmercaptopropionaldehyde with ammonia, hydrogen cyanide, and carbon dioxide in the presence of water in three reaction steps (Geiger et al., 1998).

Likewise, much of the commercially available taurine is produced synthetically by the reaction of ethylene oxide with aqueous sodium bisulfate or the reaction of aziridine with sulfuric acid. Another method involving monoethanolamine, sulfuric acid, and sodium sulfite has also been described (USDA, 2011a).

Histidine may be produced by condensing glyoxaline formaldehyde with hippuric acid (HSDB, 2002). Isoleucine is manufactured via the amination of alpha-bromo-beta-methylvaleric acid (HSDB, 2010c). Valine may be synthesized by the reaction of ammonia with alpha-chloroisovaleric acid (HSDB, 2010d).

Fermentation:

Amino acids may be produced using fermentation, the process whereby a microorganism converts nutrients to alternate components (Ajinomoto Inc., 2003; Ault, 2004). Bacterial strains that produce amino acids are primarily derived from Corynebacterium spp., Bacillus spp. or E. coli. Strains used in production are wild-type natural overproducers; auxotrophic or regulatory mutants that have altered feedback inhibition pathways or depressed enzyme synthesis; and/or genetically engineered organisms that have multiple copies of genes encoding rate-limiting enzymes (HolisticMed, undated).

During manufacture of amino acids via fermentation process, raw materials such as cane or beet molasses, raw sugar, or starch hydrolysate syrups are added to microorganism culture media, which are then allowed time to produce amino acids. Ammonia acts as the source of nitrogen and oxygen is provided by passing compressed air into the fermenting mixture (Ault, 2004). Fermentation of beet and cane sugar or starch sugars is primarily used to produce lysine for commercial use (International Starch Institute, 2012).

Enzymatic synthesis:

Amino acids may also be formed by reactions catalyzed by enzymes. The substrates may be naturally occurring, but they may also be synthetic, and are often both (Ault, 2004). During enzymatic synthesis, an amino acid precursor is converted to the target amino acid using a small number of enzymes. This manufacturing method allows the conversion to a specific amino acid without the microbial growth required in the fermentation manufacturing method (Ajinomoto Inc., 2003).
In addition, amino acids may be extracted from natural sources. During this process, hydrolysis with aqueous acid is followed by capture of the amino acids by passage of the hydrolysate over a strongly acidic ion exchange resin. The resin is washed with water and elution with aqueous ammonia is completed in order to free the amino acids. The final amino acid product is collected in fractions. Extraction is the most economical process for the production of both tyrosine and cysteine (Ault, 2004).

The processes used to manufacture the petitioned amino acids are provided in Table 4. Because this information is from 1996, the global production estimates and dominant production processes may be out-of-date.

### Table 4. Estimated Global Production of Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Production Amount (ton/year)</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>1,200</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Methionine</td>
<td>350,000</td>
<td>Chemical synthesis</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1,500</td>
<td>Extraction; Enzymatic synthesis</td>
</tr>
<tr>
<td>Lysine</td>
<td>250,000</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Taurine</td>
<td>N/A</td>
<td>Chemical synthesis</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>500</td>
<td>Fermentation; Enzymatic synthesis</td>
</tr>
<tr>
<td>Threonine</td>
<td>4,000</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Histidin</td>
<td>400</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>400</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Leucine</td>
<td>500</td>
<td>Fermentation; Extraction</td>
</tr>
<tr>
<td>Valine</td>
<td>500</td>
<td>Fermentation</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8,000</td>
<td>Fermentation; Chemical synthesis</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>120</td>
<td>Extraction</td>
</tr>
</tbody>
</table>

Source: Ikeda, 2003

**Evaluation Question #3:** Is the substance synthetic? Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

The synthetic forms of amino acids categorized as required in pet foods are petitioned for inclusion on the National List. Arginine, methionine, cysteine, lysine, taurine, tryptophan, threonine, histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine are primarily manufactured using methods of chemical synthesis and fermentation. See Evaluation Question #2 for details on methods for manufacturing synthetic amino acids. Natural forms of these substances are available and are discussed in more detail in Evaluation Question #11.

**Evaluation Question #4:** Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

Nonsynthetic amino acids exist naturally in the environment as they are commonly found in many plants, animal proteins, and animal-derived products. Please see Evaluation Question #11 for more information about natural sources of nonsynthetic amino acids.

Most amino acids present in pet foods are absorbed in the digestion system of pets. In general, animal proteins have a higher digestibility than plant proteins (NRC, 2006). Any undigested amino acids from pet food may be eliminated as nitrogenous wastes and released to the environment. Amino acids are primarily composed of nitrogen, which may persist in the environment. Nitrogen from pet waste can accumulate in the water and soil. Excess nitrogen depletes the water's oxygen, which is necessary for healthy underwater grasses, wildlife, and fish (Pennsylvania Department of Environmental Protection, undated).
The petitioned substance, synthetic amino acids used as a nutritional supplement in pet food, can enter the environment through waste streams from its production, use, and disposal. As a class of compounds, amino acids have a relatively low vapor pressure, indicating that amino acids present in soil or water are not likely to evaporate into air. Studies conducted by Gross and Grodsky (as cited in U.S. Department of Commerce National Standards Board, 1966) have indicated that most amino acids can be vaporized without decomposition. Amino acids are ionic over the entire pH range and tend to be cationic in acidic media, zwitterionic (i.e., a neutral molecule with a positive and a negative electrical charge at different locations within the molecule) in neutral media, and anionic in basic media (HSDB, 2010e).

Amino acids are considered moderately (i.e., tryptophan; HSDB, 2010b) to highly (i.e., methionine and phenylalanine; HSDB, 2010a; 2010e) mobile in soil. Amino acids are not likely to volatilize from moist soils because ions do not generally volatilize in moist soil environments. Tryptophan may not volatilize from dry soil surfaces based upon its vapor pressure (HSDB, 2010b). In addition, methionine breaks down in soil in approximately 16 days (HSDB, 2010a). The presence of amino acids in atmospheric precipitation and aerosols has been noted for many years, yet relatively little is known about these nitrogen containing organic compounds in the atmosphere (Wedyan, 2008). Amino acids can exist as a vapor or particulate in the air. Specifically, airborne methionine vapor will be degraded in the atmosphere with a half-life of about 7.5 hours. Tryptophan exists solely in the particulate phase in the atmosphere and will be removed via wet or dry deposition (HSDB, 2010b). Phenylalanine typically exists in both the particulate and vapor phases in the atmosphere. As a vapor, phenylalanine will be degraded in the atmosphere with a half-life of about 8.7 hours. Particulate-phase phenylalanine is removed from the atmosphere by wet or dry deposition, just as tryptophan (HSDB, 2010e).

Synthetic amino acids used to fortify pet food are also found naturally in water from metabolism of proteins. The potential for bioconcentration of amino acids in aquatic organisms is considered low due to its high water solubility. Synthetic amino acids, and specifically tryptophan, will degrade in water from exposure to sunlight (HSDB, 2010b). Tryptophan and phenylalanine also have an estimated bioconcentration factor (BCF) of 3, which supports the notion that the potential for bioconcentration in aquatic organisms is low. Many amino acids also lack functional groups that hydrolyze under environmental conditions (HSDB, 2010b; 2010e).

**Evaluation Question #5:** Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

All proteins in the bodies of both humans and animals are made from the same 23 amino acids. All animals convert dietary protein into tissue protein through digestive processes. Proteins are metabolized by animals through two phases: catabolism (degradation) and anabolism (synthesis). Thirteen of these amino acids can be produced by the bodies of companion animals and the remaining ten amino acids must come from dietary sources of plants and meat. NRC also considers taurine, cysteine, and tyrosine to be required amino acids for dogs and cats (Cusick, 1997). Proteins each have their own unique structure and function. The structure of amino acids varies because of their side chain which can be hydrophilic or hydrophobic. The amino acid side chain is a key factor in determining the properties of the protein made from them (Berkow, 1999). Please see the “Action of the Substance” section for more details on the functions of synthetic amino acids required in pet food for companion animals.

The toxicity of the synthetic amino acids petitioned for use in pet food varies. According to the NRC’s publication (2006) on the nutrient requirements for dogs and cats, no reports of acute or chronic toxicity related to dietary consumption of excess arginine, histidine, phenylalanine, tyrosine, threonine, tryptophan, valine, taurine, cysteine, and isoleucine in dogs were identified (2006). One report of black tongue was identified in dogs fed a diet of 12 g leucine/kg, 180 g casein/kg, and no niacin; however this observation could not be reproduced. A mechanism of antagonism was also reported for arginine and lysine and excess lysine (>40 g excess) in the diet of dogs has been associated with depression and signs of
arginine deficiency (i.e., increased plasma ammonia, emesis, etc.). Following a feeding of a single 300 g meal containing 47 g of methionine, several dogs reportedly exhibited clinical signs of toxicity including lethargy, vomiting, tremors, and disorientation. One of the dogs (aged six months) began experiencing seizures. A Safe Upper Limit (SUL) was determined to be < 47 g methionine/kg in a diet containing 4 kcal metabolizable energy (ME)/g.

No reports of clinical signs relating to acute or chronic toxicity related to large quantities of free amino arginine, histidine, isoleucine, leucine, cysteine, phenylalanine, tyrosine, valine, lysine, and threonine were identified in cats (NRC, 2006). In cats, NRC (2006) reported SULs for the following required amino acids:

- Arginine: 35 g/kg in a diet containing 4 kcal ME/g;
- Histidine: >22 g/kg in a diet containing 4 kcal ME/g;
- Isoleucine: >87 g/kg in a diet containing 4 kcal ME/g;
- Leucine: >87 g/kg in a diet containing 4 kcal ME/g;
- Lysine: >58 g/kg in a diet containing 4 kcal ME/g;
- Phenylalanine: >29 g/kg in a diet containing 4 kcal ME/g;
- Tyrosine: 68 g/kg in a diet containing 4 kcal ME/g;
- Threonine: >51 g/kg in a diet containing 4 kcal ME/g;
- Valine: >87 g/kg in a diet containing 4 kcal ME/g;
- Cysteine: no SUL provided;
- Taurine: no SUL provided.

Adverse effects associated with excess consumption of some required amino acids have been reported in cats. In mature cats, a dosage of 2 g methionine/day (20 to 30 g/kg dry diet) for 20 days induced anorexia, ataxia, cyanosis, methemoglobinemia, and Heinz body formation resulting in hemolytic anemia (NRC, 2006). The SUL for a growing kitten is estimated to be 13 g methionine/kg in a diet containing 4 kcal ME/g (NRC, 2006). One cat fed a diet of 60 g of tryptophan/kg died and was found to have interstitial fibrosis of the kidney and severe diffuse tubular degeneration and atrophy (NRC, 2006). The SUL is approximately 17 g tryptophan/kg in a diet containing 4 kcal ME/g (NRC, 2006).

The physical chemical properties of amino acids indicate that most amino acids can be vaporized without decomposition and will be moderately to highly mobile if released to soil. Please see Evaluation Question #4 for additional information on the persistence and breakdown of synthetic amino acids in the environment.

**Evaluation Question #6:** Describe any environmental contamination that could result from the petitioned substance’s manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

Pet waste containing amino acids could be released to the environment. Required amino acids contain a significant amount of nitrogen and pet wastes generally have a high nitrogen content, which can alter the physical chemical properties of amino acids and promote the growth of unwanted plant species in soil and algae in water (New Jersey Department of Environmental Protection, 2012). Water quality may also be affected because the organic matter and nutrients contained in pet waste can degrade water quality. When pet wastes decompose, the organic matter in the waste uses up dissolved oxygen and releases ammonia. Low oxygen levels and increased ammonia can kill fish (water (New Jersey Department of Environmental Protection, 2012). No information has been identified to characterize the incremental nitrogen content of pet waste, or the resulting potential for environmental effects, with the addition of synthetic amino acids to pet food.

Little data exist on the potential impact of amino acid production and use on the environment. However it is known that the manufacture of amino acids releases a significant amount of CO₂, a gas that may contribute to global warming, into the air (Ajinomoto, 2009). Air quality may be impacted in the event of an accidental release of amino acids into the atmosphere. As discussed in Evaluation Question #4, amino acids may exist in the vapor and/or particulate phase in the atmosphere.
As described in the 2001 TAP Review, synthetic production of DL-methionine involves a number of toxic source chemicals and intermediates. Each of the manufacturing processes used to produce DL-methionine was rated as either “moderately heavy” or “extreme” (Fong et al., 1981) in terms of toxics production, and it appears that newer processes have not been developed.

Methyl mercaptan, the chemical used as a catalyst in the production of methionine, can react with water, steam, or acids to produce flammable and toxic vapors (Sax, 1984). Methyl mercaptan fires are highly hazardous and can cause death by respiratory paralysis (U.S. EPA, 1987). Another potential component of methionine production is acrolein, which has a toxicity rating of 5 (on a scale of 1 to 6 with 6 being most toxic) by Gosselin et al. (1984), and it is also an aquatic herbicide (Meister, 1999). Acrolein is an eye and respiratory tract irritant (OEHHA, 2000) listed as a federal air pollutant by U.S. EPA and is 1 of 33 pollutants of “greatest concern for exposure and health effects” (U.S. EPA, 2003).

Amino acids could cause adverse effects to the environment if misused. When lysine is heated to decomposition it emits toxic fumes of nitric oxide, which could cause detrimental effects to the environment. Several material safety data sheets from taurine manufacturers state, “no data available” in the sections on ecotoxicity and environmental toxicity (Fischer Scientific, 2008; Sigma Aldrich, 2010).

However, some of the chemical intermediates used in the production of synthetic taurine could potentially impact the environment in the event of misuse or accidental release. For example, sulfuric acid can dissolve some of the soil it is spilled and can damage surrounding plants or animals exposed to it. Aziridine (also known as ethyleneimine) is flammable and reactive; it may polymerize violently when exposed to high temperatures or sunlight. It is listed as a hazardous air pollutant known or expected to cause serious health problems under the Clean Air Act (HSDB, 2006).

Disposal of amino acids into the environment should is not assumed to pose any significant risk. Many material safety data sheets for synthetic amino acids, including lysine and cysteine, advise that containers holding synthetic amino acids be “suitable” and closed containers for disposal. No further instruction for disposal is provided (Sigma Aldrich, 2012). Ajinomoto, a large manufacturer of synthetic amino acids, claims to use many of the nutrient-rich byproducts of amino acid production in their fertilizers and livestock feed (Ajinomoto Inc., 2009). The manufacturer reports that amino acids are extracted from fermentation liquors and the remaining liquid byproducts are processed into organic fertilizer (Ajinomoto Inc., 2009). Therefore the volume of waste disposed following amino acid production may be reduced if byproducts are recycled and used in other products consistent with the constructs of organic agriculture.

**Evaluation Question #7:** Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

No direct interactions between amino acids and other pet food additives were identified. The petitioned amino acids would be used in the manufacture of pet food, specifically dog and cat food. Because pets are not currently defined as “livestock” under 7 CFR 205.2, it is not anticipated that the amino acids petitioned for use in pet food would ordinarily be used with or interact with substances used in organic crop or livestock production or handling.

The primary chemical interactions of amino acids occur physiologically once inside the body. Amino acids may chemically react with one another to form peptides and eventually protein molecules. While many of the interactions associated with required amino acids may be regarded as beneficial, excess of one particular amino acid may cause deficiencies in other amino acids and induce toxicity (NRC, 2006).

However, it could be presumed that amino acid supplementation in pet food would be balanced for optimum pet health.

Methionine, while often one of the most limiting amino acids in humans, livestock, and pets, is also one that readily goes to toxic excess. In mature cats, a dosage of 2 g methionine/day (20 to 30 g/kg dry diet) for 20 days induced anorexia, ataxia, cyanosis, methemoglobinemia, and Heinz body formation resulting in...
hemolytic anemia (NRC, 2006). In addition, excess methionine exacerbates deficiencies of vitamin B6, which results in depressed growth and feed intake in poultry (Scherer and Baker, 2000).

Antagonistic relationships between required amino acids have been reported in dogs. One report of black tongue was identified in dogs fed a diet of 12 g leucine/kg, 180 g casein/kg, and no niacin; however this observation could not be reproduced. Depression and signs of arginine deficiency (i.e., increased plasma ammonia, emesis, etc.) have illustrated a possible antagonistic relationship between arginine and excess lysine in the diet of puppies (NRC, 2006). Cats are reportedly much less sensitive to arginine-lysine antagonisms and leucine-isoleucine-valine antagonisms that are commonly observed in rats (NRC, 2006).

Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil) crops, and livestock (7 U.S.C. § 6517 (c) (1) (A) (i)).

Amino acids are petitioned for use only in pet foods labeled as organic for dogs and cats. It is unlikely, that amino acids used in pet food would regularly interact with components of the agro-ecosystem. The most likely interaction would result from the release of amino acid containing pet waste near the agro-ecosystem. This interaction would not be routine and widespread, and thus is unlikely to affect agro-ecosystems.

Evaluation Question #9: Discuss and summarize findings on whether the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Little is known about the effect that amino acids have on the environment. Amino acids are a natural component of many plants and animals. Depending on the amino acid in question, and the particular process to produce it, there may be a potential for environmental impacts associated with the manufacture of these materials. The production of amino acids, such as lysine; arginine; tryptophan; threonine; isoleucine; leucine; valine; and phenylalanine by fermentation processes requires the use of a larger amount of water and energy compared with general food production practices (HolisticMed, undated). In addition, the manufacture of amino acids releases a significant amount of CO₂, a gas that may contribute to global warming, into the air (Ajinomoto, 2009).

The most likely source of possible environmental contamination associated with synthetic amino acids is through waste streams from its production. Some amino acids such as methionine are manufactured using a number of potentially toxic intermediates including methyl mercaptan and acrolein. However, it is unlikely that the use of methionine and its breakdown products will cause harm to the environment.

See Evaluation Question #4 for more information on the environmental fate and transport of synthetic amino acids added to pet food.

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

The petitioned substances are intended for use in pet foods that are not intended for human consumption and are to be added in quantities that are promote optimum animal health. Reported effects on the health of pets are more relevant and have been described in “Action of the Substance” and Evaluation Question #5. Information on reported effects of the petitioned substances on human health have been provided below; however, these effects are not necessarily expected to result from the petitioned uses (i.e., pet food nutrient supplements) of the substances. In general, we assume that amino acids would be added to pet food only at levels beneficial to animal health. Dogs, cats, and humans are generally similar in their biology (The American Physiological Society, 2001). Therefore, it can be assumed that some of the effects reported in humans could also occur in animals (The American Physiological Society, 2001), although at differing levels of exposure.
Effects upon human health have been reported for amino acids categorized as required in pet foods. However, in general, there is no evidence that amino acids derived from usual or even high intakes of protein from foodstuffs present any risk (NAS, 2009 in HSDB, 2010g). Health effects associated with the petitioned amino acids are described below:

**Arginine:**

There is evidence that the use of arginine can help humans with inborn errors of urea synthesis that have high ammonia levels in the blood and metabolic alkalosis. Arginine supplementation may also help humans with coronary artery disease, angina, or atherosclerosis, due to its effects on increasing vasodilation (blood vessel widening). Conversely, some humans may have a condition called hyperargininemia (high arginine levels in the blood), which impacts the central nervous system and can cause both behavioral and clinical changes such as ataxia (Mayo Clinic, 2011).

**Methionine:**

Methionine has been called the most toxic of amino acids in pets and humans (Garlick, 2004). Methionine may cause nausea, vomiting, dizziness, irritability, and liver dysfunction at high doses and should be used with caution in patients with severe liver disease (Reynolds, 1996). In volunteers given 10–20 g/d of methionine by mouth for 2 weeks, 7 of 11 patients with schizophrenia experienced functional psychosis (Garlick, 2004). In addition, animal studies indicate methionine may cause homocysteinemia, which is correlated with cardiovascular disease. This may be a concern for humans who use methionine as a supplement long-term (Garlick, 2004). These adverse effects are thought to be associated with the production of methanethiol-cysteine-mixed disulfides in the body.

**Cysteine:**

In humans administered 5–10-g doses of cysteine, nausea, lightheadedness, and dissociation were reported. Also, in healthy subjects given increasing doses up to 20 g of cysteine (with tranylcypromine), fatigue, dizziness, nausea, and insomnia, which were dose dependent, were reported (Garlick, 2004).

**Lysine:**

Adverse effects including renal dysfunction and failure have been reported following exposure to lysine (PDR Network, 2010 in HSDB, 2010f). Studies have reported both an increase and decrease in cholesterol levels associated with lysine administration. In human infants administered 60 to 1,080 mg of lysine monohydrochloride, no behavioral or clinical effects were observed. Similarly, no adverse effects were reported when 1- to 5-month-old infants were given up to 220 mg/kg body weight of lysine for 15 days (NAS, 2009 in HSDB, 2010f).

**Taurine:**

Therapeutic effects associated with the dietary administration have been reported. In most cases, taurine is well tolerated by humans at therapeutic doses. In one study, mild diarrhea and constipation were reported after oral taurine supplementation (Clauson et al., 2008). Another study found that taurine administered to patients with uncompensated adrenocortical insufficiency (when adrenal glands do not provide enough steroid hormones) caused hypothermia and hyperkalemia (elevated blood potassium) (Ikeda, 1996, as cited in Clauson et al., 2008). Another study reported nausea, headache, dizziness, and gait disturbances in some epileptic patients treated with 1.5 grams of taurine per day (Van Gelder et al., 1975 in Clauson et al., 2008). Taurine’s ability to conjugate bile acids, which enables the excretion of cholesterol, is one reason why taurine is thought to improve cardiovascular health. There are also a number of *in vitro*, animal, and human studies that indicate taurine may reduce blood pressure by affecting kidney vasodilation, and reducing several hormones responsible for increasing heart rate (Wójcik et al., 2010).
Tryptophan:

High dietary levels of tryptophan are associated with depressed food intake and growth in animals fed low-protein but not higher-protein diets. Behavioral effects that are mediated through serotonergic neurons, (e.g., reduced sleep latency) have been reported in animal studies; however no evidence exists of serious adverse effects attributable directly to tryptophan in humans, and some potentially beneficial effects including sleep enhancement have been reported. Tryptophan is widely sold as a sleep aid. In the 1980s, an outbreak of eosinophilia-myalgia syndrome occurred in subjects taking tryptophan supplements. The outbreak is now considered to be linked to contaminated tryptophan product rather than the tryptophan as a chemical (Garlick, 2004).

Threonine:

Threonine has been studied in low birth weight infants and in a study of 163 low birth weight infants, and an increased level of threonine in plasma may lead to increased brain glycine and affect the neurotransmitter balance in the brain which can negatively affect brain development during early postnatal life. Therefore, excessive threonine intake during infant feeding should be avoided (Boehm et al., 1998 in HSDB, 2010g). This effect is likely irrelevant in pets.

Histidine:

Histidine appears to be one of the more toxic amino acids and high dietary levels have been shown to result in potentially serious adverse effects in both animals and humans. In human studies, increases in weakness, headache, drowsiness, urinary zinc, nausea, anorexia, painful eyes, changed visual acuity, mental confusion, poor memory, and depression were observed after four overweight/obese subjects were given 24–64 g/day of histidine. However, no overt side effects were reported when up to 4.5 g/day of histidine was given as treatment for obesity, rheumatoid arthritis, and chronic uremia (Garlick, 2004).

Isoleucine:

Excess isoleucine has not been shown to have negative effects on human growth (Garlick, 2004). Isoleucine may compete with aromatic amino acids (e.g., tryptophan, histidine, etc.) for transport into brain and to lower neurotransmitter synthesis, but the significance of this effect is uncertain. Long-term studies have not provided evidence of carcinogenesis in the absence of an initiating agent (Garlick, 2004).

Leucine:

Both in vitro and in vivo studies have reported that very high doses of leucine can stimulate muscle protein synthesis, an effect that is enhanced in vivo by insulin secreted in response to the leucine dose. High levels of leucine can also inhibit protein degradation in skeletal muscle, as well as in liver. In contrast, at normal physiological levels, increasing leucine concentration by infusion stimulates muscle protein synthesis by enhancing its sensitivity to insulin. The role of leucine in vivo is to provide a signal that amino acids are available, which when combined with a signal of energy availability released by insulin, stimulates muscle protein synthesis (Garlick, 2005 in HSDB, 2010h).

Valine:

Markedly elevated concentrations of branched chain amino acids (BCAA) including valine and branched-chain alpha-keto acids are associated with maple-syrup urine disease (a hereditary metabolism disorder in which the body cannot break down certain parts of proteins). Valine imbalances appear not to cause disease and physiological abnormalities, but rather result from them (NAS, 2009 in HSDB, 2010d).

Phenylalanine:

In humans with a normal ability to metabolize phenylalanine, this amino acid is relatively safe. However, no information regarding safety during pregnancy and infancy was identified. Concern for the safety of
phenylalanine stems from the abnormal brain development known to occur in humans diagnosed with
phenylketonuria, a condition which results in the buildup of phenylalanine and its metabolites in the blood
(Garlick, 2004). No evidence that this genetic condition affects dogs and cats was identified.

**Tyrosine:**

Tyrosinemia II, a genetic disorder associated with very high plasma tyrosine levels and results in mental
retardation and lesions of the eyes and soles of the feet. However, experimental studies of high tyrosine
intake in humans have not typically reproduced these effects or the adverse effects observed in animals.
However, in babies there may be some serious health affects associated with high levels of tyrosine in the
body. A follow-up study of premature infants who had been diagnosed with transient neonatal
tyrosinemia showed an association between elevated plasma tyrosine in infancy, impaired perceptual
function, and reduced achievement scores when they reached seven years of age (Garlick, 2004).
Additionally, in a study of transient neonatal tyrosinemia attributed to a high-protein formula plus a lack
of supplemental vitamin C, children whose tyrosinemia persisted for 45 days showed lower scores on
some tests of intellectual ability. This suggests that supplemental tyrosine should be avoided by pregnant
women and infants (Garlick, 2004).

**Evaluation Question #11:** Describe all natural (non-synthetic) substances or products which may be
used in place of a petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed
substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

There are no direct substitutes for amino acids; however natural, nonsynthetic sources of amino acids do
exist. The bodies of dogs and cats can manufacture 13 of the 23 amino acids required for the creation of
proteins. The other 10 amino acids must come from dietary meat and plant sources and are called the
“essential amino acids.” There are many natural sources of the petitioned amino acids, which include the
10 amino acids that can’t be produced by the bodies of humans, dogs, and cats and three other key amino
acids. Natural (non-synthetic) sources of the 13 petitioned amino acids are identified below:

- **Arginine:** Arginine can be synthesized by the body, and is also available in pet food dietary
  sources including eggs, fish meal (tuna, white, and menhaden), poultry byproduct meal, beet
  sugar, defatted soybean flour, and in yeast (NRC, 2006).
- **Methionine:** Methionine cannot be synthesized by the body and therefore must be provided in the
  pet’s diet. Methionine is found in eggs, fish meal (tuna, white, and menhaden), milk, poultry
  byproduct meal, gluten meal, what germ, and yeast (NRC, 2006). **Cysteine:** Cysteine can be
  produced by the body from methionine. Pet food dietary sources of cysteine include beef liver,
  eggs, fish meal (tuna, white, and menhaden), poultry byproduct meal, barley grain, gluten meal,
  oats, soybean flour, wheat germ, and yeast (NRC, 2006).
- **Lysine:** Pet food dietary sources of lysine include beef (including the meat, broth, kidney, heart,
  liver, and tripe), chicken (including the meat, broth, gizzard, and liver), eggs, fish meal (tuna,
  white, and menhaden), lamb (including ground, meat, and liver), poultry byproduct meal, shrimp,
  turkey, milk, whey, beet pulp, yellow corn grain, gluten meal, oats, and rice bran (NRC, 2006).
- **Taurine:** Taurine is completely absent in cereal grains and is found in high quantities in meat
  proteins such as seafood (highest), poultry, and beef (Spitze et al., 2003).
- **Tryptophan:** Tryptophan cannot be synthesized by the body and therefore must be provided in the
  pet’s diet. Foods containing tryptophan include fish meal (tuna, white, and menhaden), poultry
  byproduct meal, milk, gluten meal, beet sugar (NRC, 2006).
- **Threonine:** Threonine cannot be synthesized by the body and therefore must be provided in the
  pet’s diet. Threonine is found in pork bacon, beef (including the meat, broth, kidney, heart, liver,
  and tripe), chicken (including the meat, broth, gizzard, and liver), eggs, fish meal (tuna, white, and
  menhaden), lamb (including ground, meat, and liver), poultry byproduct meal, shrimp, turkey,
  milk, whey. Grain barley, dried gluten feed, gluten meal, beet sugar, oats, rice bran, soybean flour,
  and wheat (Germ, germ meal, and middlings) (NRC, 2006).
- **Histidine:** Histidine cannot be synthesized by the body and therefore must be provided in the
  pet’s diet. Sources include beef (including the meat, broth, kidney, heart, liver, and tripe), chicken
(including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver),
poultry byproduct meal, shrimp, milk, turkey, yellow corn distillers, gluten feed and meal, beet sugar, and rice bran (NRC, 2006).

- **Isoleucine**: Isoleucine cannot be synthesized by the body and therefore must be provided in the pet’s diet. Isoleucine can be found in beef (including the meat, broth, kidney, heart, liver, and tripe), chicken (including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver), poultry byproduct meal, shrimp, milk, dried whey, yellow corn distillers grain, gluten meal, beet sugar, rice bran, soybean flour, wheat (flour, germ, and germ meal), and yeast (NRC, 2006).

- **Leucine**: Leucine cannot be synthesized by the body and therefore must be provided in the pet’s diet. Leucine is found in pork bacon, beef (including the meat, broth, kidney, heart, liver, and tripe), chicken (including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver), poultry byproduct meal, shrimp, milk, turkey, whey, grain barley, sugar beet pulp, yellow corn distillers, gluten meal, rice bran, sorghum grain, soybean (flour, hulls, and meal), and wheat (flour, germ, germ meal, and middlings) (NRC, 2006).

- **Valine**: Valine cannot be synthesized by the body and therefore must be provided in the pet’s diet. Sources of valine include pork bacon, beef (including the meat, broth, kidney, heart, liver, and tripe), chicken (including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver), poultry byproduct meal, shrimp, milk, turkey, grain barley, yellow corn distillers grain, yellow corn gluten meal and feed, sugar beets, oats, rice bran, soybean (flour, hulls, and meal), wheat (flour, germ, germ meal, and middlings), and yeast (NRC, 2006).

- **Phenylalanine**: Sources of phenylalanine in the diet of pets includes pork bacon, beef (including the meat, broth, kidney, heart, liver, and tripe), chicken (including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver), poultry byproduct meal, shrimp, milk, turkey, yellow corn gluten meal, sugar beet, oats, rice bran, soybean (flour, hulls, and meal), wheat (flour, germ, germ meal, and middlings), and yeast (NRC, 2006).

- **Tyrosine**: Tyrosine is a non-essential amino acid and can be made in the body using phenylalanine. Tyrosine is found naturally in beef (including the meat, broth, kidney, heart, liver, and tripe), chicken (including the meat, broth, gizzard, and liver), egg, white fish meal, lamb (ground and liver), poultry byproduct meal, milk, gluten meal, oats, rice bran, and white flower (NRC, 2006).

Although dogs and cats have been domesticated for approximately 1000 years, their digestive tract remains primarily unchanged since pre-domestication. Likewise, their protein (amino acid) requirements remain primarily unchanged from the time when the animals consumed diets consisting of killed prey. Commercial pet food brands/formulations contain different amounts of amino acids and have varying percentages of total protein. The petitioner states that “replicating the nutrient levels contained in a diet of freshly-killed game and foraged plant matter (for dogs) is extremely difficult in a commercially-manufactured food product” and notes that heat-based cooking processes decreases the bioavailability of required amino acids, specifically taurine. Therefore, additional incremental amounts of these nutrients must be added to commercially manufactured products (Pet Food Institute, 2011).

Meat and grain sources generally serve as the primary ingredients in pet food. The amino acid content most commonly found in several sources commonly used to manufacture commercial dog food is provided below in Table 5.
The petitioner reports that some histidine, isoleucine, leucine, valine, phenylalanine, and tyrosine are generally available through organically-produced dietary sources (Pet Food Institute, 2012). However, as noted by the NOSB in its November 2008 recommendation regarding Certified Organic Pet Food, arginine, methionine, cysteine, lysine, taurine, tryptophan, and threonine were included in an appendix, and believed by manufacturers to be needed in synthetic form (USDA, 2008). Because of the specific minimum (and in some cases, maximum) level of these essential nutrients that must be present in a formulated food product to be recognized as complete and balanced, small amounts of the synthetic materials are used to balance the formulations, and to compensate for the loss that occurs through the normal manufacturing process (Pet Food Institute, 2012).

Several pet food brands states that their dog food contains no synthetic amino acids, vitamins, or minerals (Carna4, 2012; Nature’s Logic, 2012). The manufacturers of Carna4 specifically state that their products use only ‘natural food ingredients to exceed all AAFCO nutrient standards,’ thereby making their products complete and balanced (Carna4, 2012). These products come in both dry and canned formulations. Therefore by using high quality ingredients, it may be possible to manufacture pet foods formulated for optimum health and performance without the inclusion of synthetic amino acids.

**Evaluation Question #12:** Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

Dogs and cats exhibit omnivorous feeding behavior and therefore their diet should be comprised of proteins, carbohydrates, fats, vitamins, minerals and water in the correct proportions. Taurine is essential for cats because they receive it from their food. The heat required during processing to meet the food safety requirements for commercially manufactured food products also decreases the bioavailability of several endogenous amino acids, including taurine. Additional incremental amounts of these amino acids may need to be added to commercially manufactured products in order to meet the nutrient standards set forth by AAFCO (Zoran, 2002 as cited in Pet Food Institute, 2012). Pet food that meets these requirements is called a “Complete” or “Balanced” diet. The most common protein added to commercial pet foods is chicken (VMRCVM, undated).

The components of commercial pet food may be processed at high temperatures for significant periods of time, which can decrease the digestibility of some amino acids that would naturally be present in meat and grain commodities (NRC, 2006). One manufacturer of pet food marketed as organic notes that their “dry foods are cooked at low temperatures to help retain important nutrients, yet provide a thorough cooking.
Canned foods are cooked under specifications set and regulated by governmental agencies to insure safety,” (Newman’s Own, 2012). Many pet owners have expressed concern with the addition of synthetic amino acids to pet food formulations because they argue that pet foods labeled as organic should contain ingredients that already have sufficient amounts of amino acids, regardless of the cooking process used by manufacturers. Additionally, the use of low quality meat byproduct “meals” as an ingredient in pet foods may supply lower amounts of amino acids for cats and dogs (NRC, 2006). Those against the use of synthetic amino acids also note that given the high quantity of amino acids naturally present in meats and grains, the use of synthetic amino acids should be unnecessary.

One alternative practice is feeding pets a raw food diet. Supporters of the raw food diet argue that dogs and cats are instinctively meat eaters and that a diet of bone, fat, raw meat, and organs represent the diet of a pet in its natural habitat. Natural proportions of vitamins, minerals, enzymes, proteins, and amino acids in raw diets can also provide wholesome nutrition for the health and well-being of pets. One commercially available product, Nature’s Variety Instinct Raw Frozen Diets, has passed AAFCO Protocol Feeding Trials and has been scientifically substantiated as complete and balanced (Nature’s Variety, 2012). Concerns with feeding pets a raw food diet include the risk of contamination from Salmonella, E. coli and other pathogenic bacteria, dietary imbalances, and internal injuries from sharp bones. Some dogs have reportedly died from bacterial poisoning (Sacks, 2010).

References:


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PDR Network, LLC. PDR for Nonprescription Drugs, Dietary Supplements, and Herbs. 31st Ed. PDR Network, LLC, Montvale, NJ. 2010 p. 596.


