Nucleotides
Handling/Processing

Identification of Petitioned Substance

Chemical Names:
Adenosine-5′-phosphate (AMP); Cytidine-5′-phosphate (CMP); Guanosine-5′-phosphate (GMP); Uridine-5′-phosphate (UMP); Inosine-5′-phosphate (IMP) (USPC, 2011)

Other Names:
AMP: Adenylic acid, Adenosine 5′-phosphoric acid; CMP: Cytidylic acid, Cytidine 5′-phosphoric acid; GMP: 5′-Guanylic acid, Disodium 5′-guanylate; UMP: Uridylic acid, Uridine 5′phosphoric acid; IMP: Inosinic acid, Inosine 5′-monophosphoric acid.

Trade Names:
AMP: Adenovite, Cardiomone, Lycedan, My-B-Den, Myoston, Phosaden (NLM, 2002). Trade names were not found for CMP, GMP, UMP, or IMP.

CAS Numbers:
AMP: 61-19-8; CMP: 63-37-6; GMP: 85-32-5; UMP: 58-97-9; IMP: 131-99-7 (NLM, 2011)

Other Codes:
AMP: EINECS* 200-500-0; CMP: EINECS 200-556-6; GMP: EINECS 201-598-8; UMP: EINECS 200-408-0; IMP: EINECS 205-045-1.
*European Inventory of Existing Commercial Chemical Substances

Characterization of Petitioned Substance

Composition of the Substance:
Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are molecules that are essential for all forms of life because they contain the cellular information needed to sustain life and growth. Both RNA and DNA are molecules made of a chain of smaller, base molecules called nucleotides (Campbell and Reece, 1996).

Nucleotides are made up of a nucleobase, a five-carbon sugar, and a phosphate group. Nucleobases are nitrogen-containing molecules that fall into two classes: pyrimidines and purines. Pyrimidines are made up of one six-atom ring containing carbon and nitrogen. Cytosine and uracil are the pyrimidine nucleobases in RNA, while DNA contains cytosine and thymine. Purines also contain a six-atom ring made of carbon and nitrogen, but that ring is also attached to a five-atom carbon and nitrogen ring. Guanine and adenine are the purine nucleobases in RNA and DNA. The purine nucleobase inosine is found in RNA (Campbell and Reece, 1996). The petitioned nucleotides include only those nucleotides found in RNA.

The petitioned nucleotides all have a phosphate group attached to the fifth carbon of the pentose, which is called the 5′ position. Four of the five petitioned nucleotides are the typical nucleotides in RNA: adenine 5′-phosphate (AMP), guanosine 5′-phosphate (GMP), cytidine 5′-phosphate (CMP), and uridine 5′-phosphate (UMP). The fifth nucleotide is inosine 5′-phosphate (IMP), a nucleotide that is a precursor in the synthesis two other nucleotides, AMP and GMP and can sometimes be incorporated into RNA (Lehninger et al., 1993; Campbell and Reece, 1996). The chemical structures of the five petitioned nucleotides are presented in Figure 1.

Nucleotides play important roles in many specific biological processes, including cellular signaling, and are constituents of biologically important coenzymes, molecules required for the normal functioning of cellular enzymes. Nucleotides are considered "conditionally essential" nutrients. This means that they are not normally required in the diet, but must be supplied to some individuals during certain disease states because those individuals cannot synthesize the compound internally, or their need for the compound is greater than their capacity for synthesis (Fürst and Stehle, 2004). This may be especially true of nucleotides during periods of rapid growth or in the case of some diseases affecting infants (Singhal et al., 2010).

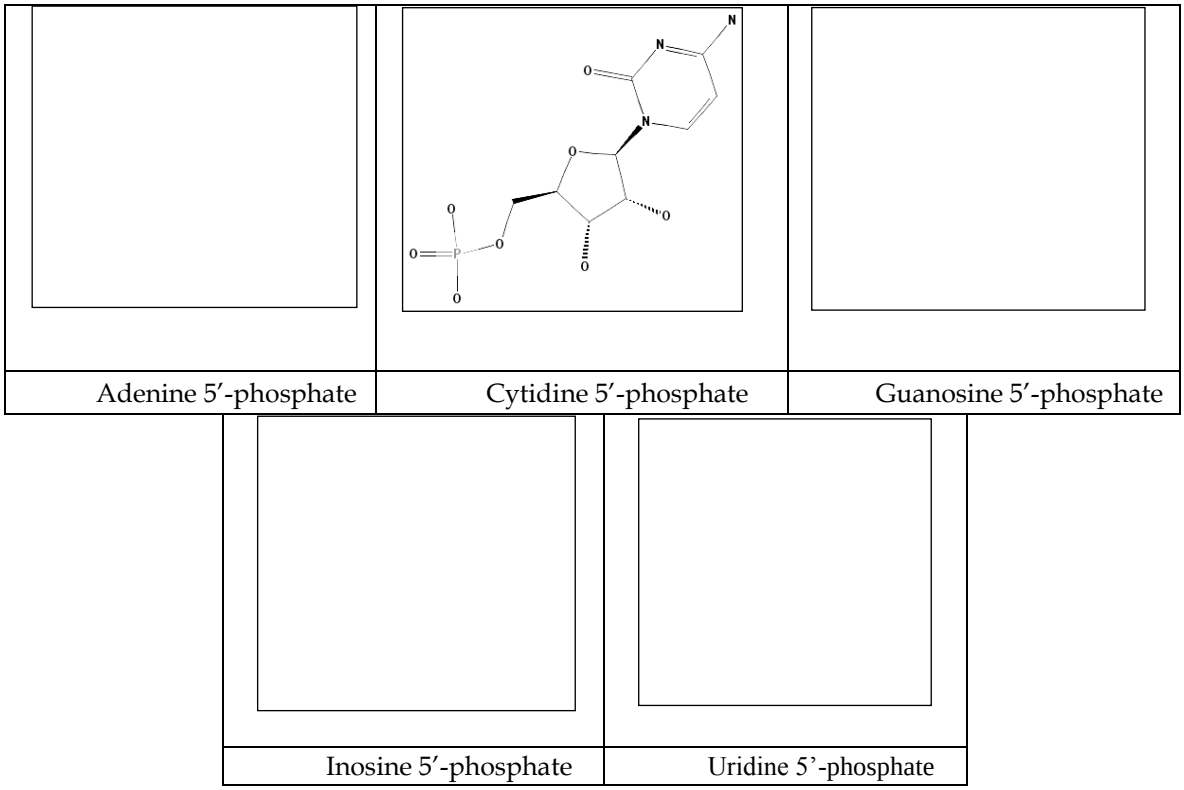

Figure 1: Chemical Structures of the Petitioned Nucleotides (NLM, 2012)

Properties of the Substance:

The physical and chemical properties of the petitioned nucleotides are presented in Table 1. The properties presented in Table 1 describe each of the individual nucleotides, as indicated.

Table 1. Chemical Properties of Petitioned Nucleotides

Property	AMP	CMP	Disodium GMP	Disodium UMP	Disodium IMP
CAS #	61-19-8	63-37-6	5550-12-9	3387-36-8	4691-65-0
Color	Colorless or white	Colorless or white	Colorless or white	Colorless or white	Colorless or white
Physical State	Crystals or crystalline powder	Crystals or crystalline powder	Crystals or crystalline powder	Crystals	Crystals or crystalline powder
Molecular Weight	347.23	323.20	407.18 (Sigma-Aldrich, 2011)	368.15	392.17
Odor	Odorless (Zhen-Ao Group, 2009a)	Odorless (Zhen-Ao Group, 2009b)	Odorless (Zhen-Ao Group, 2009c)	Odorless (Zhen-Ao Group, 2009d)	Odorless (Yamasa Corporation, 2007)
Melting Point	196-200 °C (384.8-392 °F) (NLM, 2002)	222 °C (432 °F) (Sigma-Aldrich, 2009)	NA	208-210 °C (406-410 °F) (Sigma-Aldrich,	NA

Property	AMP	CMP	Disodium GMP	Disodium UMP	Disodium IMP
				2008)	
Boiling Point	NA	NA	NA	NA	NA
Solubility	Readily soluble in boiling water, soluble in 10% hydrochloric acid, insoluble in alcohol (NLM, 2002)	Slightly soluble in water, practically insoluble in alcohol. (USPC, 2011)	Soluble in water, practically insoluble in ether, and sparingly soluble in alcohol. (USPC, 2011)	NA	Freely soluble in water (Yamasa Corporation, 2007)
Stability	Free acids and salts are stable for long periods in dry state; neutral solutions also stable (NLM, 2002)	Stable under recommended storage conditions. (Sigma-Aldrich, 2009)	NA	Recommended storage temperature: -20 °C (Sigma-Aldrich, 2008)	NA
Reactivity	Stable (Zhen-Ao Group, 2009a)	Stable (Zhen-Ao Group, 2009b)	Stable (Zhen-Ao Group, 2009c)	Stable (Zhen-Ao Group, 2009d)	Stable under storage conditions (Yamasa Corporation, 2007)
Flammability	Flash point: 67° C (NLM, 2002), may be combustible at high temperatures (Sciencelab.com, 2010)	NA	NA	NA	NA
Hazardous Combustion/ Decomposition	Combustion products are carbon oxides and nitrogen oxides (Sciencelab.com, 2010)	None found, but likely similar to AMP and Disodium IMP	None found, but likely similar to AMP and Disodium IMP	None found, but likely similar to AMP and Disodium IMP	Toxic fumes of carbon monoxide, nitrogen oxides, carbon dioxide (Yamasa Corporation, 2007)

Table data from USPC, 2011 unless otherwise listed. NA=Not Available

Specific Uses of the Substance:

Nucleotides are found in abundance in the diets of adults and weaned infants, and are also found in human breast milk. Nucleotides are one of the nutrient groups that may contribute to the observed biological benefits for children consuming breast milk. Nucleotides serve important biochemical functions in the body, including functioning as mediators of physiologic processes, precursors to nucleic acid synthesis, sources of cellular energy, and constituents of coenzymes (Carver, 2003).

The amount of nucleotides in human breast milk is higher than that in both dairy-based and soy-based infant formula, so nucleotides are added as a supplement to those infant formulas to raise the content to the levels in human milk (Carver, 2003; Ostrom et al., 2002; Singhal et al., 2010). The petitioned use of the substance, as stated by the petitioner, is to fortify infant formula with nucleotides from yeast RNA hydrolysate to the level of nucleotides provided by human breast milk (International Formula Council, 2011).

The same nucleotides obtained for use in supplementing infant formula have an historical use in food as flavoring agents. In a patent from Sakaguchi et al. (1965), the authors note that nucleotides can be used to flavor soups, meat products, sauces, curry powder, dressings, and various drinks, including wine. The patent authors suggest that the taste enhancing properties of the nucleotides is caused by a synergistic reaction between the 5'-nucleotides and amino acids in the foods (Sakaguchi et al., 1965).

Nucleotides may also be used as a dietary supplement for people with specific conditions. Due to evidence that nucleotides may aid in the growth and development of cells with rapid turnover such as gastrointestinal cells, nucleotide supplements have been used by people with Irritable Bowel Syndrome (IBS). Results of a randomized, controlled trial showed that nucleotide supplementation improved some symptoms of IBS (Dancey et al., 2006).

Nucleotides have been used for experimental purposes as a dietary supplement in animal feed in both agriculture and aquaculture (e.g. farming fish and shrimp). The research trials evaluated immune response, growth, and tissue development of the livestock after treatment with nucleotides. Experimental livestock used in nucleotide supplementation studies include: pigs, cattle, tilapia, chicken, and shrimp. Nucleotides were added to both dry feed and liquid feed (milk) in these applications (Hoffman, 2007a, 2007b, 2007c). Nucleotides are not permitted as feed additives by the U.S. Food and Drug Administration or the American Association of Feed Control Officials.

Approved Legal Uses of the Substance:

Nucleotides do not currently appear on the USDA National List of Allowed and Prohibited Substances (hereafter referred to as the National List) for use in handling/processing of organic food for human consumption. Nucleotides are not a required nutrient in infant formula, according to 21 CFR 107(d), therefore no levels of nucleotides have been specified by FDA as required in infant formula. Nucleotides were originally thought to fall under the "Nutrient Vitamins and Minerals" classification in the National List. As of January 2012, this interpretation has been clarified and updated such that nucleotides and other additives to organic products not specified in the rule must be petitioned by the manufacturer and reviewed on an individual basis. See the section on "OFPA, USDA Final Rule" for further information on the status of nucleotides in organic handling.

AMP is the subject of a FDA Agency Response Letter to the industry proposed GRAS Notice No. GRN 000144. In the notice, the proposed use of AMP is as a flavor enhancer in various foods, drinks, chewing gum, and sweeteners at levels ranging from 0.0002 percent to 0.0008 percent (FDA, 2004). The Agency does require labeling with a specific common name (adenosine 5'-monophosphoric acid or adenosine 5'-monophosphate) since it is considered a flavor enhancer, not a flavor, and cannot be labeled as "natural flavor". The maximum recommended therapeutic dose (MRTD) for AMP is 0.333 mg/kg bodyweight per day (FDA, 2009). None of the other petitioned nucleotides were listed in the MRTD database.

Nucleotides can be used legally as a human dietary supplement, but they are not required to be registered with the FDA for this use. The FDA does not regulate human dietary supplements in the same way as drugs or animal feed additives. Generally speaking, manufacturers do not need to register their products with FDA or receive approval before producing and selling supplements for human consumption. However, the product manufacturer is responsible for ensuring the safety of the product and notifying FDA that they will be marketing the product before the product is sold. Paperwork for a product named "ReaL Build" manufactured by Natural Source International, Ltd. was filed in 1997 as a dietary supplement

containing nucleotides extracted from *Escherichia coli* bacteria. 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).

Action of the Substance:
Nucleotides serve as mediators of physiologic processes, precursors to nucleic acid synthesis, sources of cellular energy, and constituents of coenzymes. They are found in abundance in the diets of adults and weaned infants, and are also found in human breast milk. Internal synthesis of nucleotides is costly from a metabolic standpoint and nucleotides are more efficiently obtained from either the diet or by a salvage pathway, which is a way the body recycles nucleobases or nucleotides when DNA or RNA is broken down (Lehninger et al., 1993; Carver, 2003). Nucleotides are one of the nutrient groups that may contribute to the observed biological benefits for children consuming breast milk (Singhal et al., 2010).

Rapidly developing tissues in infants, including the lymph, gastrointestinal, and blood cell systems, have an increased need for nucleotides in order to function optimally. This need cannot be met through internal synthesis of the nucleotides, so dietary sources of nucleotides are necessary. The absence of an exogenous supply of nucleotides will not cause a clinical deficiency, but nucleotides are needed to maximize functioning of the developing systems (Singhal et al. 2010).

Nucleotide-supplemented diets have been shown to increase immune responses in laboratory animals (Carver, 2003). In another study, infants fed nucleotide-supplemented diets had enhanced antibody responses to influenza and diphtheria (Pickering, 1998 as cited in Carver, 2003). Other studies have observed increased immune system responses, growth, and weight gain in infants following nucleotide-supplemented diets (Singhal, 2010; Carver, 2003).

Combinations of the Substance:
The petitioned nucleotides are obtained from yeast RNA hydrolysate, according to the petitioner. Yeast is on the National List (section 205.605(a)) as a nonsynthetic, nonagricultural substance that is allowed to be used in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))." Thus, yeast is a precursor to the petitioned substance, in that yeast is hydrolyzed to produce nucleotides. The National List includes four types of yeast: bakers, brewers, nutritional, and smoked yeasts, as well as yeast autolysate. As described under Evaluation Question #1, commercial preparation of nucleotides are commonly derived from fresh baker's yeast. The National List states that yeast is not allowed to be used if it has been grown on petrochemical substrates or sulfite waste liquor (7 CFR §205.605(a)).

The petitioned nucleotides are petitioned for addition to organic infant formula. Organic infant formula contains a number of nutrients (e.g., riboflavin, niacin, pantothenic acid, iodine, copper, potassium) included on the National List (7 CFR 205.605), which identifies nutrient vitamins and minerals allowed for use in organic products as those in the FDA Nutritional Quality Guidelines for Food (21 CFR 104.20(d)(3)). The NOP recently published a proposed rule that would amend the National List reference to 21 CFR 104.20. In particular, the proposed amendment would specify that vitamins and minerals are allowed in organic infant formula as required by 21 CFR 107.100 or 107.10 (USDA, 2012), which is FDA's regulatory standard for infant formula. Various food ingredients comprising carbohydrates, proteins, fats, and stabilizers are expected to be included in infant formula to which the petitioned nucleotides are added. These ingredients vary with the type of product and manufacturer.

Status

Historic Use:
A patent for the production of "humanized milk" enriched with the nucleotides AMP, CMP, GMP, IMP and UMP was filed in 1982 and registered in 1985. (Gil and Valverde, 1985) The patent describes efforts to understand the nucleotidic composition of human breast milk for use in the production of infant formula dating back to 1952. (Gil and Valverde, 1985) A patent exists for the production of 5-nucleotides from yeast extract, as described by Sakaguchi and Kuninaka (1965), for use as flavoring agents. The process generates

all of the 5'-nucleotides discussed in this report, despite the fact that the end use in the patent is different that the focus of this report. No information was found on the historic use of nucleotides in organic agricultural handling or processing.

OFPA, USDA Final Rule:
The petitioned substance is not explicitly described in the OFPA or USDA Final Rule, but was initially, incorrectly interpreted to fall within the listing at 7 CFR §205.605(b), "Nutrient vitamins and minerals, in accordance with 21 CFR 104.20, 'Nutritional Quality Guidelines for Foods'" in the National List. A previous, incorrect NOP interpretation of the Nutritional Quality Guidelines for Foods allowed for the use of "accessory nutrients." The term "accessory nutrients" was used by NOP to indicate substances that are Generally Recognized as Safe (GRAS) and are added to infant formula, but are not classified as essential vitamins or minerals by the FDA, nor required by FDA regulations. The NOP interpretation of the FDA guidelines essentially allowed an exemption for nutrient vitamins and minerals added to certain foods, including infant formula.

A proposed rule published January 12, 2012 in the Federal Register (77 FR 1980) aimed to update the National List and correct the initial interpretation regarding nutrient vitamins and minerals. The proposed rule, if implemented, would limit the vitamins and minerals that can be added to organic infant formula to those vitamins and minerals considered essential by FDA and the vitamins and minerals that are specifically required by FDA to be added to infant formula (21 CFR §101.9, 21 CFR §107.100, 21 CFR §107.10). Nucleotides are not considered essential vitamins and minerals, nor are they on the list of vitamins and minerals required by the FDA to be added to infant formula. Under the proposed rule, ingredients such as nucleotides must be petitioned to the NOSB and approved for use in organic infant formula before they can be added.

International:
No information was available from the Canadian General Standards Board (CGSB) on nucleotides used in organic infant formula. Nucleotides are included in the CGSB Draft Organic Aquaculture Standards as "Feed, Feed Additives, and Supplements" (CGSB, 2011).

Nucleotides including AMP, CMP, GMP, UMP, and IMP are included in the Advisory List of Nutrient Compounds for Use in Foods for Special Dietary Uses Intended for Infants and Young Children published by the Codex Alimentarius Commission (CAC, 2009). In the Report of the 28th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, the Delegation of Mexico and other delegations supported establishing a maximum level of nucleotides in infant formula at 16 mg per 100 kCal. Still other delegations proposed leaving the establishment of specific levels up to national authorities, and the Committee agreed, stating that levels may need to be determined at a national scale (CAC, 2007).

The European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008 does not list nucleotides in general or the individual nucleotides specifically. However, Article 20 of the EEC Council Regulation No 834/2007 of 28 June 2007 states that organic yeast may be used as a food or feed only if the yeast is produced on organically produced substrates. It does state that other products may be used as yeast substrates if they have been otherwise approved by the Council Regulation for use in organic production (EEC, 2007) .

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: 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 process described by the petitioner involves extracting nucleotides from RNA in fresh bakers' yeast by way of enzymatic hydrolysis, a process by which an enzyme (phosphodiesterase derived from bacteria) is used to break down RNA into its constituent parts, the 5'-nucleotides. Yeast extract is a rich source of RNA,

which is in turn a rich source of 5′-nucleotides. Yeast extract rich in RNA is heated to 95 °C for ten minutes to inactivate yeast enzymes that would cause autolysis, when the yeast's own digestive enzymes break down their own proteins into simpler compounds.

The yeast cells must then be lysed (broken down) to release the RNA, which requires treatment with the enzyme protease at 62 °C. The protease enzyme is then inactivated by raising the temperature to 70 °C, after which the pH is lowered to 5.3 with hydrochloric acid. To obtain RNA from the resulting mixture, the solids (yeast cell wall material) and liquids (RNA, proteins, carbohydrates, minerals, lipids, and vitamins) are separated by centrifugation and filtered using ultrafiltration to remove vitamins, carbohydrates, and low-molecular weight amino acids. The extracted RNA is hydrolyzed, or broken into smaller pieces, by a water molecule with the help of the phosphodiesterase enzyme obtained from the bacterium *Penicillium citrinum*. Hydrolysis occurs at the phosphodiester bond in the RNA chain, a process which yields the individual nucleotides from the original chain of nucleic acids, after incubation for 15 hours (Noordam and Kortest, 2011).

The resulting hydrolysate, the product of the hydrolysis process, must then be filtered using ion-exchange resin columns and separated by chromatography (a laboratory technique that separates mixtures) to yield four of the individual nucleotides: AMP, CMP, GMP, and UMP. The remaining nucleotide, IMP, is a breakdown product of AMP which can also be generated by treating AMP with the enzyme deaminase (Noordam and Kortest, 2011). The nucleotides are processed and prepared for packaging using filtration, crystallization, centrifugation, drying, sieving, milling, and blending (International Formula Council, 2011).

Additional accounts of the process from other sources are useful for the sake of comparison. A patent by Sakaguchi and Kuninaka (1965) describes a similar method using one of a number of strains of bacteria (including *Penicillium* species), yeasts, and molds that produce the 5′-phosphodiesterase enzymes necessary for nucleotide extraction.

Descriptions of yeast processing using autolysis, a naturally occurring process, are readily available. However, the enzymatic hydrolysis process is necessary in this case, as yeast autolysis does not generate 5′-nucleotides, but rather produces nucleosides and nucleic acids. Usually the yeast extract is heated to denature the yeast's native enzymes to prevent autolysis, as described above (EURASYP, 2011).

The process of obtaining nucleotides from yeast, as described by the petitioner, is one of stepwise extraction and purification. Nucleotides are the constituent parts of RNA, RNA is a constituent of cellular proteins, and cellular proteins are a constituent of yeast cells, which are agglomerated in yeast extract. The main feedstock in this process is yeast. Multiple chemical changes are made to the yeast in order to extract nucleotides, including heating to denature proteins, cell wall proteolysis, enzymatic hydrolysis, and dehydration. All of these processes are used to obtain the nucleotides contained in the yeast, which are not themselves chemically changed, but rather refined and extracted using the described chemical processes (Noordam and Kortest, 2011; International Formula Council, 2011; EURASYP, 2011).

Evaluation Question #2: 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)).

As discussed in Evaluation Question #1 above, 5′-nucleotides are obtained by treating yeast extract with various chemical processes that do not occur naturally, including heating, the addition of acids to lower the pH of solutions, proteolysis, and enzymatic hydrolysis. Enzymes used in the process are obtained from natural sources, but the extraction of the enzymes requires non-natural processes. The phosphodiesterase enzyme is derived from *P. citrinum*, a natural fungal source (Epicentre, 2011). Proteinase K, a protease, is an enzyme that works on a broad spectrum of proteins and was originally isolated from the fungus *Engyodontium album*, a natural source. The petitioner does not name the source of the protease enzyme used in their process, but Proteinase K has been used for RNA extraction from yeast (Worthington Biochemical, 2012; Sigma-Aldrich, 2012). Though the phosphodiesterase enzyme that the petitioner uses is obtained from *P. citrinum*, other microbiological sources are described (Sakaguchi and Kuninaka, 1965), as are plant

sources including malt rootlets from malted barley, a waste product of the brewing industry. (Sombutyanuchit et al., 2001)

Both of the necessary enzyme treatments hydrolyze bonds through enzymatic action, a process that occurs in natural systems. The enzymes are obtained from naturally-occurring, microbiological sources. However, it is unlikely that both enzymatic processes necessary to extract nucleotides from RNA in yeast would occur in nature, and certainly not at the scale necessary for commercial production. In addition, the yeast must be treated with heat to prevent the autolysis that would occur naturally, as well as acid treatment to adjust the pH of the intermediate solutions. The further purification of the nucleotides after extraction also may involve ion-exchange resin columns for filtration and centrifugation, which are not naturally occuring processes (Sakaguchi and Kuninaka, 1965; Sombutyanuchit et al., 2001; Noordam and Kortest, 2011).

Evaluation Question #3: Provide a list of non-synthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

Nucleotides are naturally-occurring substances and can either be obtained from the diet or synthesized in the human body. Nucleotides are found in all known forms of life, but they are not readily available because the source of nucleotides, RNA, exists mostly within the ribosomes of the cell (Campbell and Reece, 1996). Thus, any RNA found in living things is natural or non-synthetic, but non-natural processes are required to release RNA and then refine the RNA into its constituent nucleotides.

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status. What is the technical function of the substance?

AMP is the subject of a FDA Agency Response Letter, to the industry proposed GRAS Notice No. GRN 000144. In the notice, the proposed use of AMP is as a flavor enhancer in various foods, drinks, chewing gum, and sweeteners at levels ranging from 0.0002 percent to 0.0008 percent (FDA, 2004). The Agency Response states that FDA has no objection to the use of AMP under the conditions of use specified in the notice (FDA, 2004). GRAS listings were not available for the other nucleotides. As discussed in the "Specific Uses" section, the technical function of the substance (nucleotides) is to fortify infant formula with nucleotides to a level similar to that provided by human breast milk (International Formula Council, 2011).

Evaluation Question #5: Describe whether the primary function/purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

The primary function of the petitioned substance is as a nutritional supplement in infant formula. The petitioned substance serves as a source of dietary nucleotides, which function as physiologic mediators, precursors for the formation of nucleic acids, sources of cellular energy, and constituents of coenzymes (Campbell and Reece, 1996). As much as 5 percent of dietary nucleotides are incorporated into tissues from the diet, with the highest amounts incorporated in times of limited food intake or rapid growth (Carver, 2003). Thus, the primary function/purpose of the petitioned substance is not as a preservative.

Evaluation Question #6: Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

As proposed by the petitioner, nucleotides would be added to infant formula to improve nutritive value of the formula (International Formula Council, 2011). The nutrient deficit that exists in dairy- and soy-based formulas, which the petitioned substance has been used to correct, is not due to processing. Rather, dairy- and soy-based formulas are naturally lacking in nucleotides at the levels observed in breast milk. The intent of using the petitioned substance is to supplement the formula with nucleotides up to a level consistent with breast milk. The added nucleotides are not required by any regulations.

Evaluation Question #7: Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

The addition of nucleotides to infant formula increases the nutritional quality of the formula by providing a nutrient normally found in human breast milk. As discussed in the "Action of the Substance" section, nucleotides play an important role in physiological processes and maintenance. Nucleotides are normally found in human breast milk, but not in dairy- and soy-based infant formulas (Carver, 2003). Nucleotides are one of the nutrient groups that may contribute to the observed biological benefits for children consuming breast milk. A randomized controlled trial suggested that adding nucleotides to infant formula led to increased weight gain and head growth (Singhal et al., 2010). Nucleotide-supplemented diets have also been shown to increase immune responses in laboratory animals (Carver, 2003). In another study, infants fed nucleotide-supplemented diets had enhanced antibody responses to influenza and diphtheria (Pickering, 1998 as cited in Carver, 2003).

Evaluation Question #8: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

No information was available on residues of heavy metals or other contaminants in excess of FDA tolerances in nucleotides. No action levels were found related to nucleotides or yeast extract in FDA's Action Levels for Poisonous or Deleterious Substances in Human Food. In the National List (section 205.605(a)), it is stated that yeast is not allowed to be used if it has been grown on petrochemical substrates or sulfite waste liquor, which could potentially lead to contamination of the yeast extract. The Food Chemicals Codex (USPC, 2011) lists the following acceptance criteria for impurities in the individual nucleotides:

Table 2. Acceptance Criteria for Impurities in Nucleotides

	Arsenic (Inorganic)	Cadmium	Lead	Mercury	Ammonium Salts
AMP	< 2 mg/kg	< 0.1 mg/kg	< 1 mg/kg	< 0.5 mg/kg	-
CMP	< 2 mg/kg	< 0.1 mg/kg	< 1 mg/kg	< 0.5 mg/kg	-
Disodium GMP	-	-	< 5 mg/kg	-	Litmus test not blue
Disodium IMP	-	-	< 5 mg/kg	-	Litmus test not blue
Disodium UMP	< 2 mg/kg	< 0.1 mg/kg	< 1 mg/kg	< 0.5 mg/kg	-

Source: USPC (2011)

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

The petitioned substances are found in all living cells. The manufacture of the substance is carried out using yeast in controlled environments (e.g., bakeries, breweries, or dedicated yeast fermentation facilities) where the yeast is made to grow and multiply. Once the yeast is obtained and yeast extract is generated, refining and separation of the nucleotides involves laboratory practices that are carried out in controlled environments. Any environmental contamination resulting from purification and extraction of the nucleotides in laboratory settings would likely be subject to regulations governing waste discharges from the laboratories. Yeast that are cultured for baking or brewing processes would not likely survive outside of the environment for which they have been bred, and are unlikely to have environmental impacts.

Once the substances are incorporated into infant formula, they are likely to be ingested by infants or disposed of when the infant formula is past its expiration date. It is not likely that disposal of the expired

infant formula would have an adverse effect on the environment given that food waste, including milk and soy, is common in landfilled refuse. In addition, the amounts of nucleotides included in infant formula make up less than 2 percent of the total ingredients by weight, as indicated on the labels provided by the petitioner (International Formula Council, 2011).

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 most serious health effects related to nucleotides would likely result from a lack of nucleotides in the diet or from a physiological inability to synthesize nucleotides within the body. The nucleotides discussed in this report are essential to normal cell function, and the majority of nucleotides in the human body are synthesized within the body. Up to 5 percent of the nucleotides obtained from the diet are incorporated into tissues (Carver, 2003).

As discussed in Evaluation Question #7, above, nucleotide-supplemented diets have been shown to increase immune responses in laboratory animals (Carver, 2003). In another study, infants fed nucleotide-supplemented diets had enhanced antibody responses to influenza and diphtheria (Pickering, 1998 as cited in Carver, 2003). Other studies have observed increased immune system responses, growth, and weight gain in infants following nucleotide-supplemented diets (Singhal, 2010; Carver, 2003).

Evaluation Information #11: Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b)(1)).

No information was found on organic alternatives for the production of nucleotides. As discussed previously, nucleotides exist in all cells (Campbell and Reece, 1996), but the extraction process required to release them is necessarily synthetic because it involves refining yeast extract to the constituent nucleotides in the yeast cells' RNA. These processes require specific enzymatic treatments and physical treatments such as centrifugation and filtration (Noordam and Kortest, 2011; International Formula Council, 2011; EURASYP, 2011).

Several varieties of organic yeast are available, so organic yeast could be used as an organic alternative feedstock for the production of organic nucleotides (OMRI, 2012). It is possible that organic yeast used in the production of certified organic bread or beer could be employed. However, hydrochloric acid and synthetic heating and filtering processes must be used in the nucleotide extraction process, as described in Evaluation Question #2. Hydrochloric acid is not on the National List, and therefore the process would be disqualified from organic certification.

References:

CAC. 2009. Advisory List of Nutrient Compounds for Use in Foods for Special Dietary Uses Intended for Infants and Young Children. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program. CAC/GL10-1979. Amended 2009.

CAC. 2007. Report of the 28th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program. ALINORM 07/30/26 Rev.

Campbell, N. A. and J. B. Reece. 1996. Biology (ed.). Benjamin/Cummings series in the life sciences.

Carver, J. D. 2003. Advances in nutritional modifications of infant formulas. The American journal of clinical nutrition **77**:1550S.

CGSB. 2011. Can/Cgsb-32.311-2006: Organic Production Systems Permitted Substances Lists. Canadian General Standards Board. Retrieved December 15, 2011 from http://www.ocpro.ca/docs/standards/PSL%20032-0311-2008-eng.pdf

Dancey, CP; Attree, EA; Brown, KF. 2006. Nucleotide supplementation: a randomised double-blind placebo controlled trial of IntestAidIB in people with Irritable Bowel Syndrome. Nutrition Journal, 5: 16.

Epicentre. 2011. MasterPure™ Yeast RNA Purification Kit. Epicentre Biotechnologies, Madison, WI. Retrieved on December 15, 2011 from http://www.epibio.com/pdftechlit/207pl0611.pdf

EURASYP. 2011. Yeast Extract with Natural Nucleotides. European Association for Specialty Yeast Products. Paris, France. Retrieved on January 11, 2012 from http://www.yeastextract.info/public/documents/yeast-products/yeast_extract_with_natural_nucleotides.pdf

EEC. 2007. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. European Economic Community. Luxembourg.

FDA. 2009. Maximum Recommended Therapeutic Dose (MRTD) Database. Retrieved January 11, 2012 from: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm092199.htm

FDA. 2005. Dietary supplements. Retrieved January 25, 2012 from: http://www.fda.gov/food/dietarysupplements/default.htm.

FDA. 2004. Agency Response Letter GRAS Notice No. GRN 000144. Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Food and Drug Administration. Retrieved November 7, 2011 from: http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASListings/ucm153976.htm

Fürst, P; Stehle, P. (2004). What are the essential elements needed for the determination of amino acid requirements in humans? Journal of Nutrition 134 (6 Suppl): 1558S-1565S.

Gil, A. and L. Valverde. 1985. Nucleotide enriched humanized milk and process for its preparation. U.S. Patent Number 4,544,559. Assignee: Union Industrial y Agroganadera, S.A. (UNIASA). Retrieved on January 10, 2012 from http://www.google.com/patents/US4544559.

Hoffman, K. 2007a. The Use of Nucleotides in Animal Feed, Part 1. FEED-MIX, 15(4): 25-27. Retrieved February 24, 2012 from www.chemoforma.com/uploads/1203673317375.pdf

Hoffman, K. 2007b. The Use of Nucleotides in Animal Feed, Part 2. FEED-MIX, 15(5): 14-16. Retrieved February 24, 2012 from www.chemoforma.com/uploads/1203673456422.pdf

Hoffman, K. 2007c. The Use of Nucleotides in Animal Feed, Part 3. FEED-MIX, 15(6): 36-38. Retrieved February 24, 2012 from www.chemoforma.com/uploads/1203673564407.pdf

International Formula Council. 2011. Petition to Include Nucleotide-Rich Yeast Autolysate at 7 CFR 205.605. International Formula Council, Atlanta, GA.

Lehninger, AL; Nelson, DL; Cox, MM. (1993). Principles of biochemistry (Second Edition ed.). New York: Worth Publishers, Inc.

NLM. 2012. PubChem Database. National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD. Retrieved January 10, 2012 from http://pubchem.ncbi.nlm.nih.gov/.

NLM. 2011. ChemIDplus Lite – Adenosine 5'-monophosphate. TOXNET, Toxicology and Environmental Health Information Program, U.S. National Library of Medicine, Bethesda, MD. Retrieved December 11, 2011 from http://chem.sis.nlm.nih.gov/chemidplus/.

NLM. 2002. Hazardous Substances Data Bank - Adenosine 5'-Phosphate. TOXNET, Toxicology and Environmental Health Information Program, U.S. National Library of Medicine, Bethesda, MD. Retrieved December 22, 2011 from http://toxnet.nlm.nih.gov/

Noordam, B. and J. G. Kortest. 2011. Production of 5'-ribonucleotides. U.S. Patent Application Number 20060078972. Nixon and Vanderhye, PC. Arlington, VA

OMRI. 2012. OMRI Brand Name Products List and Generic Materials List, Organic Materials Review Institute. Eugene, Oregon. Retrieved on March 7, 2012 from http://www.omri.org/simple-gml-search/results/yeast.

Ostrom, K. M., Cordle, C.T., Schaller, J.P., Winship, T.R., Thomas, D.J., Jacobs, J.R., Blatter, M.M., Cho, S., Gooch III, W.M., Granoff, D.M. 2002. Immune status of infants fed soy-based formulas with or without added nucleotides for 1 year: part 1: vaccine responses, and morbidity. Journal of Pediatric Gastroenterology and Nutrition, 34: 137-144.

Sakaguchi, K. and A. Kuninaka. 1965. Production of 5'-nucleotides. U.S. Patent Number 3,223,592. Yamasa Shoyu Co., Ltd. Choshi, Japan.

Sciencelab.com. 2010. Material Safety Data Sheet – Adenosine-5′-monophosphate. Sciencelab.com, Inc. Retrieved on December 15, 2011 from http://www.sciencelab.com/msds.php?msdsId=9922804

Sigma-Aldrich. 2012. Material Safety Data Sheet - Proteinase K, from *Tritirachium album*. Sigma-Aldrich Co., LLC. Retrieved on January 11, 2012 from http://www.sigmaaldrich.com/catalog/

Sigma-Aldrich. 2011. Material Safety Data Sheet - Guanosine 5′-monophosphate disodium salt hydrate. Sigma-Aldrich Co., LLC. Retrieved on January 11, 2012 from http://www.sigmaaldrich.com/catalog/

Sigma-Aldrich. 2009. Material Safety Data Sheet - Cytidine 5′-monophosphate disodium salt. Sigma-Aldrich Co., LLC. Retrieved on January 11, 2012 from http://www.sigmaaldrich.com/catalog/

Sigma-Aldrich. 2008. Material Safety Data Sheet - Uridine 5′-monophosphate disodium salt. Sigma-Aldrich Co., LLC. Retrieved on January 11, 2012 from http://www.sigmaaldrich.com/catalog/

Singhal, A., K. Kennedy, J. Lanigan, H. Clough, W. Jenkins, A. Elias-Jones, T. Stephenson, P. Dudek, and A. Lucas. 2010. Dietary Nucleotides and Early Growth in Formula-Fed Infants: A Randomized Controlled Trial. Pediatrics 126:e946.

Sombutyanuchit, P., M. Suphantharika, and C. Verduyn. 2001. Preparation of 5′-GMP-rich yeast extracts from spent brewer's yeast. World Journal of Microbiology and Biotechnology **17**:163-168.

USPC. 2011. Food Chemicals Codex Monographs. First Supplement, FCC 7. U.S. Pharmacopeial Convention, pgs. 317-319, 1431-1434, 1449-1452, 1462-1465.

Worthington Biochemical. 2012. Proteinase K. Worthington Biochemical Corporation, Lakewood, NJ. Retrieved on January 11, 2012 from http://www.worthington-biochem.com/PROK/default.html.

Yamasa Corporation. 2009. Material Safety Data Sheet, Inosine 5′-monophosphate, disodium salt. Yamasa Corporation, Chiba-ken, Japan. MSDS provided by petitioner.

Zhen-Ao Group. 2009a. Material Safety Data Sheet, Adenosine-5′-monophosphate free acid. Zhen-Ao Group Co., Ltd. Dalian, China. MSDS provided by petitioner.

Zhen-Ao Group. 2009b. Material Safety Data Sheet, Cytidine-5′-monophosphate free acid. Zhen-Ao Group Co., Ltd. Dalian, China. MSDS provided by petitioner.

Zhen-Ao Group. 2009c. Material Safety Data Sheet, Guanosine-5′-monophosphate disodium salt. Zhen-Ao Group Co., Ltd. Dalian, China. MSDS provided by petitioner.

Zhen-Ao Group. 2009d. Material Safety Data Sheet, Uridine-5′-monophosphate disodium salt. Zhen-Ao Group Co., Ltd. Dalian, China. MSDS provided by petitioner.