Petition to Include Nucleotides isolated from Yeast RNA Hydrolysate on the National List of Substances Allowed as Ingredients in or on Processed Products Labeled as "organic" or "made with organic (specified ingredients or food group(s))."

ITEM A

Section of the National List:	§ 205.605. Non-agricultural (non-organic) substances allowed as ingredients in or on processed products labeled as ''organic'' or ''made with organic (specified ingredients).''
Specific Listing:	"Nucleotides from Yeast RNA Hydrolysate, identified as the following, and their sodium salts:
	Adenosine-5'-phosphate (AMP)
	Cytidine-5'-phosphate (CMP)
	Guanosine-5'-phosphate (GMP)
	Uridine-5'-phosphate (UMP)
	Inosine-5'-phosphate (IMP)

ITEM B

1. The chemical or common names of the substance.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) consist of long polymers of simple units called nucleotides, with backbones made of five-carbon sugars and phosphate groups joined by ester bonds. Attached to each sugar is one of four types of molecules called bases or nucleobases. Another term, nucleo**side**, is used in nucleic acid chemistry. A nucleo**side** is a molecule consisting of a base and a five-carbon sugar (i.e., containing no phosphate). A nucleo**tide** is a molecule with three moieties: a nucleobase, a five-carbon sugar, and one or more phosphate groups.

The two chemical differences between **R**NA and **D**NA include the five-carbon sugar group – **r**ibose or **deoxy**ribose – and one of the four bases. The bases adenine, cytosine, and guanine are found in both DNA and RNA, thymine is found only in DNA, and uracil is found only in RNA.

The table below shows the common names of the nucleoside and nucleotide of each RNA base. The specific nucleo**tides** used to supplement infant formulas contain ribose (ribonucleotides) and have the phosphate group attached at the 5' position of the ribose molecule.

Nucleo Base	Nucleoside	Ribonucleotide relevant to infant formula
Adenine	Adenosine	Adenosine-5'-phosphate (AMP)
Cytosine	Cytidine	Cytidine-5'-phosphate (CMP)
Guanine	Guanosine	Guanosine-5'-phosphate (GMP)
Uracil	Uridine	Uridine-5'-phosphate (UMP)
Hypoxanthine	Inosine*	Inosine-5'-phosphate (IMP)*

*Inosine is a normal physiological metabolite of adenosine.

Common or usual name	CAS & EPA Registry Names	
Adenosine-5'-phosphate	5'-Adenylic acid	5'-Adenylic acid, sodium salt
Cytidine-5'-phosphate	Cytidine monophosphate (5'–Cytidylic Acid USP)	
Guanosine-5'-phosphate	5'-Guanylic acid	Disodium 5'-guanylate
Uridine-5'-phosphate	Uridine monophosphate	Uridine, 5'-dihydrogen phosphate, disodium salt
Inosine-5'-phosphate	Inosinic acid	5'-Inosinic acid, sodium salt (1:2)

2a. The petitioner's name, address and telephone number and other contact information.

International Formula Council
1100 Johnson Ferry Road NE, Suite 300
Atlanta, GA 30342.
Contact: Mardi Mountford, Executive Vice President
Phone: (678) 303-3027
Email: mmountford@kellencompany.com

2b. Sources of the petitioned substance (Nucleotides from Yeast RNA Hydrolysate)

Two commercial sources of the individual nucleotides are Yamasa Corporation, Biochemicals Division, and Dalian Zhen-Ao-Bio-Tech Co., Ltd.:

1. Yamasa Corporation
10-1 Araoicho 2-Chome Choshi,
Chiba-ken 288-0056, Japan
Tel: 81-3-3668-0311 / Fax: 81-3-3668-0312
website: http://www.yamasa.com/english/bio/index2.html

2. Dalian Zhen-Ao Bio-Tech Co., Ltd.
NO. 88 Life First Rd.
DD Port, Dalian, China, 116620
Tel: 86 (0411) 3924-8033 / Fax: 86 (0411) 3924-8233
Website: http://bio.zhen-ao.com
email: trade@zhen-ao.com.

3. Current Use.

Nucleotides originating from yeast hydrolysate are currently used as nonagricultural ingredients to fortify conventional infant formulas and infant formulas labeled as "organic," in accordance with the recommendations of independent professional associations and International standards for infant formula.

The specific function of these nucleotides is as "nutrient supplements" [21 CFR 170.3(o)(20)].

4. Handling activities for which the substance is used.

Nucleotides from Yeast RNA Hydrolysate are added to infant formula products to fortify them to the level of nucleotides provided by human milk.

Mode of action:

Nucleotides form the basis of DNA and RNA, are important in tissues with rapid turnover (for example, the gut and immune system), and are metabolic regulators (involved in energy transfer, 'acid' handling, synthesis and breakdown of large molecules.) They may act as growth factors and may have immunomodulating effects on immune defenses.

A large clinical study[1] published in 2010 in the journal *Pediatrics* documented that infants fed nucleotide-supplemented formula had greater head circumference at ages 8, 16, and 20 weeks than infants fed control formula. Weight at 8 weeks and the increase in both head circumference and weight from birth to 8 weeks were also greater in infants fed nucleotide-supplemented formula than in those fed control formula. The conclusion of the study was that nucleotides could be conditionally essential for optimal infant growth in some formula-fed populations.

[1] Dietary nucleotides and early growth in formula-fed infants: a randomized controlled trial**.** Singhal A, Kennedy K, Lanigan J, Clough H, Jenkins W, Elias-Jones A, Stephenson T, Dudek P, Lucas A. Pediatrics. 2010 Oct;126(4):e946-53. Epub 2010 Sep 13.

5. Source of the substances and a detailed description of its manufacturing process.

The specific material added to infant formula to achieve the desired levels of the individual nucleotides – AMP, CMP, GMP, IMP, and UMP – is a mixture of the individual 5'-nucleotides isolated from 5'-nucleotide-rich yeast RNA hydrolysate produced with phosphodiesterase enzyme (CAS No. 54576-84-0) from *Penicillium citrinum*, a non-GMO organism.

Yeast RNA is the starting material for manufacture of 5'-nucleotides. Fresh bakers' yeast contains 6 to 8 % RNA, but special strains may contain 13 % or more (all figures refer to dry solids contents). The RNA in torula yeast and/or brewers' yeast is dissolved in purified water by steam heat, and hydrolyzed by the enzyme phosphodiesterase (CAS No. 54576-84-0) from *Penicillium citrinum* at controlled temperature and pH for about 12 hours. The hydrolysate is then passed through ion-exchange resin columns and is eluted chromatographically to obtain the CMP, AMP, UMP, and GMP in sequence. The four 5'-nucleotides are collected in separate tanks and undergo nanofiltration, aseptic filtration, crystallization, centrifugation, drying, sieving, milling, blending, and packaging.

The 5'-nucleotide of inosine (IMP) arises as a normal breakdown product of AMP or can be produced by treating AMP with the enzyme deaminase.

The individual 5'-nucleotides are combined in a desired ratio or added individually to create the desired proportions of each nucleotide in an infant formula.

6. Summary of historical reviews of the petitioned substance.

a. Life Sciences Research Office (LSRO), American Societies for Nutritional Sciences. Assessment of Nutrient Requirements for Infant Formulas; J Nutr 1998; 128(Supp):2059S–2298S. (under contract for the FDA).

According to the summary of the LSRO report reviewing the nutrients in infant formula (p. 2060), "the specification of a maximum value for . . . nucleotides . . . , in conjunction with a minimum value of zero, did not constitute an endorsement for the inclusion of that substance; but rather a recognition of apparent safety at levels defined by the maximum. Additional rationale for each nutrient is provided in the 'Conclusions and Recommendations' sections."

This additional rationale of this 1998 report reads as follows (p. 2067S):

> ***Nucleotides***
>
> ***Minimum:*** **The Expert Panel found no compelling reason to require the addition of nucleotides to infant formulas at this time.** Preliminary evidence of beneficial effects from nucleotide supplementation of infant formulas is intriguing, and the Expert Panel strongly urges continued research in this area. When data from long-term, large-scale clinical trials are available, the question of addition of nucleotides to infant formulas should be reconsidered, preferably within five years.

> ***Maximum:*** **The Expert Panel recommended a maximum content of nucleotides and nucleotide precursors in infant formula of 16 mg/100 kcal, a value similar to the upper limit reported for human milk.** The Expert Panel specified that the maximum level of free nucleotides, including available nucleosides, nucleic acids (DNA and RNA) that serve as nucleotide precursors be limited to the amount present in human milk and not exceed 20% of the total nonprotein nitrogen supplied in infant formula and a maximum level of 16 mg/100 kcal.

The 1998 LSRO recommendation for a maximum content of nucleotides and nucleotide precursors (polymeric nucleic acids) in term infant formula was 16 mg/100 kcal, a value similar to the upper limit of nucleotide equivalents reported for human milk. The European recommendations are for far lower amounts.

b. Scientific Committee on Food. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae; Brussels, European Commission 2003; SCF/CS/NUT/IF/65 Final 2003; Pages 60-64:

> ***4.7.3 Nucleotides and nucleosides***
>
> Nucleosides contain a nitrogenous base and a pentose, but no phosphate group. Nucleotides contain a nitrogenous base, a pentose and one or more phosphate groups. Nucleotides are found primarily intracellularly. They are the structural components of DNA and RNA.
>
> Nucleotides such as adenosine triphosphate (ATP) transfer chemical energy. Other nucleotides are involved in the synthesis of proteins, lipids and carbohydrates (e.g. nicotine adenine dinucleotide, NAD; flavin adenine dinucleotide, FAD).
>
> Human milk contains free ribonucleosides and ribonucleotides (Gil and Sanchez-Medina, 1982; Topp *et al.,* 1993; Leach *et al.,* 1995; Schlimme and Schneehagen, 1995) (Table 15). In addition, human milk contains RNA and DNA, of which the major part is located within cells. Leach *et al.* (1995) reported data for "total potentially available nucleosides" released from previously frozen human milk samples after sequential treatment with sodium hydroxide, nuclease, pyrophosphatase, phosphatase and phosphoric acid. This method should detect also nucleotides hydrolysed from polymeric ribonucleotides (RNA and DNA) and adducts, and includes intracellular nucleosides. Leach and coworkers reported markedly higher concentrations of "total potentially available nucleosides", as compared to the reported amounts of free ribonucleosides and ribonucleotides (Table 15). Of the "total potentially available nucleosides", 48±8% were reported to be polymeric nucleotides, 36±10% monomeric nucleotides, 8±6% nucleosides and 9±4% adducts (nucleoside-phosphatephosphate-X, e.g. uridine diphosphate galactose or NAD) (Leach *et al.,* 1995). However, it is not known which proportion of the "total potentially available nucleosides" in human milk is utilised by the breast fed infant in vivo. While human milk nucleotides might exert beneficial effects in the breast-fed infant, it is also possible that nucleotides and nucleosides, RNA and DNA might occur in human milk only as a by-product of milk formation that reflect metabolic activity of the mammary gland tissue, shedding of somatic cells and occurrence of microorganisms, without

having a specific function for the infant. Accordingly, higher concentrations of ribonucleosides in colostrum than in mature milk have been interpreted as a consequence of the high metabolic activity of the mammary gland during the first days postpartum, along with higher contents of cellular components (Schlimme *et al.*, 2000).

Table 15. Reported mean contents of ribonucleosides, ribonucleotides, and so-called "total potentially available ribonucleosides" in mature human milk (mg/L)

	Adenine	Cytosine	Guanine	Uracil	Total
Ribonucleotides[1]	7.8	7.0	1.2	4.8	
Ribonucleosides[2]	0.4-1.8	1.0-1.2	0.1-0.3	0.1-1.7	
"Total potentially available nucleotides"[3]	10.6	29.3	6	12.6	67.2-67.5

[1] Gil and Sanchez-Medina, 1982
[2] Topp *et al.*, 1993; Leach *et al.*, 1995; Schlimme and Schneehagen, 1995
[3] Leach *et al.*, 1995

Nucleotides and nucleosides are synthesised *de novo* in human metabolism, and nucleotides liberated during nucleic acid catabolism can be reutilized via a salvage pathway. Hence nucleotides and nucleosides are considered to be dispensable nutrients. In breast-fed infants the dietary intake of preformed nucleotides accounts for only a minor fraction of the requirement. Uauy *et al.* (1994) estimated that only about 2% of nucleotide accretion in growing tissues of the infant is covered by the ingestion of nucleotides with breast milk. Even if the sum of nucleotides and nucleic acids in breast milk could be utilized by the infant they would account for only about 15% of nucleotide accretion (Uauy *et al.*, 1994). Thus, the infant must be capable of effectively synthesizing nucleotides. Moreover, the assumption that the sum of nucleotides and nucleic acids in breast milk could be utilized may not be correct.

In human adults, the effect of ingested free nucleotides on urinary uric acid excretion indicates practically complete absorption of purines from such sources, whereas dietary RNA increases urinary uric acid excretion to a lesser extent, hence dietary RNA is not fully absorbed and utilised (PAG *Ad hoc* Working Group, 1975). The effect of DNA on uric acid levels is only about half of that of RNA (PAG *Ad hoc* Working Group, 1975). Human infants fed a formula based on soybean protein isolate, which contains relatively large amounts of soy RNA, were reported to have serum uric acid concentrations near the upper end of the reference range, suggesting that infants utilise soy RNA at least in part (Kuchan *et al.*, 2000). However, a systematic evaluation on the relative utilisation of free nucleotides, RNA and DNA in infants, for example with a comparison of their effects on uric acid excretion, is not available. In any case based on the quantitative considerations cited above it is unlikely that the nucleotide supply provided with human milk has marked effects on tissue accretion.

Even if dietary nucleotides, nucleosides, RNA and DNA would have little systemic effects, they may be utilised by some tissues such as intestinal mucosa cells. It should also be noted that the addition of nucleotides to the food provides an added source of NPN, and of phosphorus, which under certain circumstances might have relevant metabolic effects.

Several clinical trials have been presented that aim at evaluation of effects of nucleotide addition to formula in infants, but only two of the trials have studied formulae with nucleotide levels of 72 mg/L (Pickering *et al.*, 1998, Lasekan *et al.*, 1999).

Carver *et al.* (1991) studied 15 healthy term infants randomised to a formula without and 13 infants to a formula with 33 mg/L free nucleotides, as well as a reference group of 9 breast fed babies. There were no differences in clinical outcomes such as growth or infection rates. Natural killer cell activity and interleukin 2 production of peripheral blood mononuclear cells in vitro was significantly higher in infants fed formula with nucleotides than the control formula at the age of 2 months, but not at 4 months. The clinical relevance of this difference is not known.

Brunser *et al.* (1994) studied infants from a lower socioeconomic group in Chile fed formulae without (n=148) and with (n=141) 14.2 mg free nucleotides/100 g powder, which would yield a nucleotide concentration of about 2 mg/100 mL product as ready to feed. During the study period of 3 months, the group fed formula with nucleotides experienced a significantly lower number of first episodes of diarrhoea (74 *vs.* 102). No difference in the spectrum of enteropathogens found in stools and in growth parameters was found.

Martínez-Augustín *et al.* (1997 a and b) evaluated formula supplementation with about 11.6 mg nucleotides/L relative to a control diet in preterm infants. Lactose/mannitol ratios indicating intestinal permeability as well as serum concentrations of β-lactoglobulin were not different. Serum IgG antibodies to β-lactoglobulin on day 30 were higher in the nucleotide supplemented group, whereas antibodies to alpha-casein did not differ. The clinical relevance of this difference is not known.

Cosgrove (1998) studied infants born small for gestational age fed a formula with about 33 mg nucleotides/L (n=39) or a control formula without added nucleotides (n=35). Supplemented infants showed a greater gain in weight, length and head circumference between birth and 2 months, as well as between birth and 6 months (e.g. weight gain 0-6 months 80.1 *vs.* 71.8 g/week and kg weight at baseline, p=0.05).

Pickering *et al.* (1998) performed a multi-center trial with one year duration in term infants. The study was completed by 101 infants fed formula with 72 mg/L nucleotide, 107 infants fed a control formula, and 124 infants fed human milk. There were no differences between the formula groups with respect to indicators of growth and tolerance. Infants fed nucleotide supplemented formula had higher antibody titres to HiB and diphtheria at 7 months of age. Data on the incidence of diarrhoea were monitored only at 2 of the 13 study sites. The evaluation of the data from these two sites indicated a lower number of infants that suffered from at least one episode of diarrhoea (15 *vs.* 41%, $p<0.05$).

Lasekan *et al.* (1999) followed infants fed formula with 72 mg/L nucleotides (n=138) or unsupplemented control (n=147), of which about 80% completed the one year study. There were no group differences in growth, tolerance, adverse effects, the incidence of diarrhea, illness visits to physicians, antibiotic prescription, and antibody responses after vaccination to HiB and diphtheria, but anti polio virus type 1 titres were higher in the nucleotide group. Immune phenotype analysis showed an increased proportion of memory lymphocytes and a reduced proportion of naive lymphocytes in the supplemented group. The clinical relevance of these differences is not known.

The available studies show some indication for a possible modulation of immune phenotypes and antibody responses to vaccination by the addition of free nucleotides to infant formulae in healthy term babies. There are some indications for a potential protective effect against diarrhoea in compromised, and possibly also in healthy infants. An enhancing effect on growth has only been reported in infants born small for gestational age, in comparison to a control formula apparently not matched in nitrogen and phosphorus contents. No dose effect between nucleotide concentrations used and outcomes has been shown. There are no indications for relevant adverse effects of nucleotide addition in the concentrations tested.

The Infant Formulae Directive has approved the addition of nucleotides to infant formulae and follow-on formulae in concentrations up to 1.5 mg/100 kcal 5'-AMP, 2.5 mg 5'-CMP/100 kcal, 0.5 mg 5'-GMP/100 kcal, 1.75 mg 5'-UMP/100 kcal, 1.00 mg 5'-IMP/100 kcal, and a total concentration of up to 5 mg/100 kcal, which is similar to reported data for free ribonucleotides in human milk (about 4-6 mg/100 kcal).

The Protein-Calorie Advisory Group of FAO/WHO has recommended an upper limit of 2 g/day for the addition of nucleic acids to the diet of human adults (PAG *Ad hoc* Working Group 1975). Based on 70 kg body weight, this amount would be equivalent to an intake of about 28.6 mg/kg. In infants consuming a formula intake providing 100 kcal per kg and day with the current maximum level of 5 mg/100 kcal would thus be equivalent to a maximal daily nucleotide intake of 5 mg/kg. If the maximum level would be increased to 16 mg/100 kcal, the estimated daily nucleotide intake of an infant fed such formula would be 16 mg/kg and thus rather close to the recommended adequate level of intake set for adults. However, there are no data to indicate that this level of intake would have adverse effects in infants. No studies are available that evaluate a dose-response relationship between the concentrations of nucleotides in infant formula and relevant outcomes in infants. Thus, there is no adequate scientific basis at present to conclude that the addition of nucleotides in higher concentrations than presently permitted for infant formula would provide additional benefits.

In the absence of evidence of benefit of increasing the levels of added nucleotides permitted at present in infant formulae, the Committee recommends that the content of nucleotides, if added to infant formulae and in follow-on formulae, should not exceed 5 mg/100 kcal. If added the maximum nucleotide contents should be: cytidine 5'-monophosphate (CMP) 2.5 mg/100 kcal, uridine 5'-monophosphate (UMP) 1.75 mg/100 kcal, adenosine 5'-monophosphate (AMP) 1.50 mg/100 kcal, guanosine 5'-monophosphate (GMP) 0.50 mg/100 kcal, inosine 5'-monophosphate (IMP) 1.00 mg/100 kcal. Formula based on soy protein isolates should be excluded from the option of further addition of nucleotides because of their high natural contents.

c. Global Standard for the Composition of Infant Formula: Recommendations of an ESPGHAN Coordinated International Expert Group. ESPGHAN Committee on Nutrition. Journal of Pediatric Gastroenterology and Nutrition, 41:584–599 November 2005. Page 596:

> *Nucleotides*
>
> Several publications have reported beneficial effects of the addition of nucleotides to infant formulae (2,3). The IEG did not find sufficient data to support additional benefits from increasing intakes to levels greater than 5 mg/100 kcal, while adverse effects of higher contents such as increased risk of respiratory tract infections have been reported (65). The optional addition of nucleotides at a maximum total content of 5 mg/100 kcal as well as maximal levels of 2.5 mg/100 kcal CMP, 1.75 mg/100 kcal UMP, 1.5 mg/100 kcal AMP, 0.5 mg/100 kcal GMP, and 1.0 mg/100 kcal IMP are recommended.

d. The European Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, the current European regulation for infant formula composition, provides for the addition of nucleotides to infant formula, as follows:

> ANNEX I - ESSENTIAL COMPOSITION OF INFANT FORMULAE WHEN RECONSTITUTED AS INSTRUCTED BY THE MANUFACTURER
>
> The values set out in this Annex refer to the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer.
>
> 12. NUCLEOTIDES
>
> The following nucleotides may be added:

	Maximum (1)	
	(mg/100 kJ)	(mg/100 kcal)
cytidine 5′-monophosphate	0.60	2.50
uridine 5′-monophosphate	0.42	1.75
adenosine 5′-monophosphate	0.36	1.50
guanosine 5′-monophosphate	0.12	0.50
inosine 5′-monophosphate	0.24	1.00

> (1) The total concentration of nucleotides shall not exceed 1.2 mg/100 kJ (5 mg/100 kcal).

e. The Pediatric Nutrition Handbook, 6th Edition, published by the Committee on Nutrition, American Academy of Pediatrics, in 2009, at page 810, states:

> **Nucleotides**
>
> Nucleotides (components of RNA and DNA) normally are found in human milk at concentrations of approximately 189±70 µmol/L . Currently, nucleotides are added to several infant formulas in the United States. The mechanism by which dietary nucleotides may modify immune function is unknown, although recent mouse-model studies indicate they ma6 augment T helper 1-biased immune responses. Studies in human infants have reported that adding nucleotides to infant formula increases natural killer cell activity, IL-2 production by monocytes, serum IgM and IgA concentrations, and serum antibody titers to food antigens. The clinical relevance of these changes is unknown. Two studies have reported more clinically specific endpoints. One study showed higher antibody titers to H. influenzae type b vaccine in treated infants, and another study reported a reduced duration of diarrheal disease in a group of children of low socioeconomic status. Such data are promising, but additional studies are needed to understand the mechanism of action, confirm clinical endpoints, and monitor the long-tem effects of adding nucleotides to infant formula.

7. Information regarding the regulatory status of Nucleotides.

The National Organic Program regulation at 7 CFR 205.605(b) lists "Yeast – nonsynthetic, growth on petrochemical substrate and sulfite waste liquor is prohibited (Autolysate; Bakers; Brewers; Nutritional; and Smoked – nonsynthetic flavoring process must be documented)."

The yeast RNA hydrolysate used to provide nucleotides in infant formulas is made from yeast RNA extracted from naturally occurring yeast grown on acceptable substrates and is chemically changed only by naturally occurring biological processes using a natural enzyme from a non-GMO organism.

The FDA allowed the supplementation of infant formulas with nucleotides in the late 1990s in accordance with the process mandated by the Infant Formula Act of 1980, amended in 1986.

The European Community has approved the nucleotide acids and sodium salts of AMP, GMP, IMP, CMP (cytidine 5'-monophosphate) and UMP (uridine 5 '-monophosphate) as food additives that may be added for specific nutritional purposes in foods for particular nutritional uses. The European Union Infant Formula Directive permits, but does not require, addition of nucleotides.

The Food Chemicals Codex created monographs for these five 5'-nucleotides in 2010. These monographs are found in Appendix A.

8a. Chemical Abstract Service (CAS) Numbers of the Nucleotides and their Sodium Salts.

	CAS No.		
	Nucleotide	Sodium salt	comment
Adenosine-5′-monophosphate AMP	61-19-8	13474-03-8	
		149022-20-8	the hydrate
Cytidine-5′-monophosphate CMP	63-37-6		
Guanosine-5′-monophosphate GMP	85-32-5	5550-12-9	
Uridine-5′-monophosphate UMP	58-97-9	3387-36-8	
Inosine-5’-monophosphate IMP	131-99-7	4691-65-0	

8b. Labels of products that contain nucleotides.

See Appendix B (Labels).

9. The substances’ physical properties and chemical mode of action.

The Food Chemicals Codex in 2010 submitted to FDA monographs on the individual nucleotides prepared by enzymatic hydrolysis of yeast RNA, for use in infant formula. These Food Chemical Codex monographs, attached in Appendix A, describe the physical properties of the five 5’-nucleotides.

Chemically, nucleotides are the building blocks of RNA, molecules essential to life. Infants fed infant formulas supplemented with these nucleotides show greater head circumference (brain) growth and somatic growth compared to infants fed unsupplemented infant formulas.

From a National List perspective, 5’-monophosphate nucleotides are a special form of yeast autolysate. The original Brewers yeast from which the RNA has been separated is a nutritious and totally biodegradable material that can be used for livestock feed. As the original 1995 TAP Reviewers of yeast autolysate concluded, there is minimal risk of environmental contamination during the manufacture, use, misuse, or disposal of yeast autolysate, and yeast autolysate is compatible with a system of sustainable agriculture.

10a. Safety information about the substance including a Material Safety Data Sheet (MSDS).

The MSDS sheets for 5’-nucleotides produced by enzymatic hydrolysis of yeast RNA are attached in Appendix C.

10b. National Institute of Environmental Health Studies Substance Report.

A specific NIEHS report on yeast 5'-nucleotides does not exist, to our knowledge.

11. Research information about Nucleotides.

The 1998 LSRO report referred to above asked that "when data from long-term, large-scale clinical trials are available, the question of addition of nucleotides to infant formulas should be reconsidered, preferably within five years."

In 2004, two papers reported the effect of nucleotide supplementation on the immune response of infants from birth through 12 months and demonstrated positive changes in T-cell and serum antibody responses when infants were fed nucleotide-supplemented formula:

> Schaller et al. **(**Schaller, J.P.; Kuchan, M.J.; Thomas, D.L.; Cordle, C.T.; Winship, T. R.; Buck, R.H.; Baggs, G.E.; & Wheeler, J.G. **Effect of dietary ribonucleotides on infant immune status. part 1: humoral responses.** Pediatr. Res. 2004; 56 (6) :883-890) and
>
> Buck et al. (Buck, R.H.; Thomas, D.L.; Winship, T.R.; Cordle, C.T.; Kuchan, M.J.; Baggs, G.E.; Schaller, J.P.; & Wheeler, J.G. **Effect of dietary ribonucleotides on infant immune status. Part 2: immune cell development.** Pediatr. Res. 2004; 56 (6) :891-900)

More importantly, Gutiérrez-Castrellón et al (2007) conducted a systematic review of the available evidence of nucleotide supplementation in infants. Their findings are summarized in the Abstract below:

Immune response to nucleotide-supplemented infant formulae: systematic review and meta-analysis. Pedro Gutiérrez-Castrellón, Ignacio Mora-Magaña, Luisa Díaz-García, Carlos Jiménez-Gutiérrez, Jaime Ramirez-Mayans and Guillermo A Solomon-Santibáñez. Br J Nutr 2007 98, Suppl. 1, S64–S67.

> Human milk is recommended as the only alimentary source for the first six months of life. Additionally there is a medical and social need for safe and effective alternative forms of nutrition for infants who cannot be fed with breast milk. Recently the safety and efficacy of some ingredients in infant formulae, such as nucleotides have been discussed. This systematic review analyzed the available evidence to establish the efficacy, safety and dose-response effect of ribonucleotide-supplemented infant formulae (RSIFs). Randomised controlled clinical trials (RCTs) comparing RSIFs to formulae without nucleotides or breast milk were considered in this review. Outcome measures were: antibody titres to common paediatric vaccinations, total lymphocytes, lymphocyte subclasses and NK-cells, episodes of diarrhoea and acute respiratory infection. Publication quality was determined using Jadad and CONSORT guidelines. Results were combined using a random effects model and reported through standardized mean differences (WMD) or risk ratio (RR). Systematic review and meta-analysis showed that RSIFs were associated with a better antibody response to immunisation with *Haemophilus influenzae* vaccine [SMD 1·74 (99 %CI 1·43–2·05), P ¼ 0·001], diphtheria toxoid [SMD 0·94 (0·75–1·12), P ¼ 0·001], oral polio vaccine [SMD 0·73

(0·51–0·95), P ¼ 0·001], and fewer episodes of diarrhoea [RR 0·67 (0·58–0·76), P ¼ 0·02]. We did not find a major risk of upper respiratory infections [RR 1·11 (0·90–1·36), P ¼ 0·50]. Available evidence suggests a positive benefit of RSFIs on infant health without any risk. These benefits begin with nucleotide addition of 1·9 mg/418·4 kJ and are maintained or increased with 10·78 mg/418·4 kJ.

The most significant recent news about nucleotide supplementation of infant formula is the results of a clinical study published in 2010, in the journal *Pediatrics*, which documented that nucleotide supplementation lead to increased weight gain and head growth in formula-fed infants. The details of this clinical trial are as follows:

Dietary nucleotides and early growth in formula-fed infants: a randomized controlled trial. Singhal A, Kennedy K, Lanigan J, Clough H, Jenkins W, Elias-Jones A, Stephenson T, Dudek P, Lucas A. Pediatrics. 2010 Oct;126(4):e946-53. Epub 2010 Sep 13.

Abstract

Background: Dietary nucleotides are nonprotein nitrogenous compounds that are found in high concentrations in breast milk and are thought to be conditionally essential nutrients in infancy. A high nucleotide intake has been suggested to explain some of the benefits of breastfeeding compared with formula feeding and to promote infant growth. However, relatively few large-scale randomized trials have tested this hypothesis in healthy infants.

Objective: We tested the hypothesis that nucleotide supplementation of formula benefits early infant growth.

Patients and Methods: Occipitofrontal head circumference, weight, and length were assessed in infants who were randomly assigned to groups fed nucleotide-supplemented (31 mg/L; n=100) or control formula without nucleotide supplementation (n=100) from birth to the age of 20 weeks, and in infants who were breastfed (reference group; n=101).

Results: Infants fed with nucleotide-supplemented formula had greater occipitofrontal head circumference at ages 8, 16, and 20 weeks than infants fed control formula (mean difference in z scores at 8 weeks: 0.4 [95% confidence interval: 0.1-0.7]; P=.006) even after adjustment for potential confounding factors (P=.002). Weight at 8 weeks and the increase in both occipitofrontal head circumference and weight from birth to 8 weeks were also greater in infants fed nucleotide-supplemented formula than in those fed control formula.

Conclusions: Our data support the hypothesis that nucleotide supplementation leads to increased weight gain and head growth in formula-fed infants. Therefore, nucleotides could be conditionally essential for optimal infant growth in some formula-fed populations. Additional research is needed to test the hypothesis that the benefits of nucleotide supplementation for early head growth, a critical period for brain growth, have advantages for long-term cognitive development.

The Life Sciences Research Office (LSRO), American Societies for Nutritional Sciences, published the Assessment of Nutrient Requirements for Infant Formulas. J Nutr 1998;

128(Supp):2059S–2298S. (under contract for the FDA). The extensive and balanced discussion of nucleotides in this document (pages 2122S-2125S) is available in Appendix D.

12. Petition Justification Statement.

Cows' milk contains considerably lower amounts of nucleotides and their exact chemical composition differs from those in human milk. Due to degradation during heat treatment, many infant formulas contain low concentrations of nucleotides so that supplements are added during the manufacture of some infant formulas to provide levels of nucleotides comparable to those of human milk. The recommendations from the Life Sciences Research Office (LSRO) under their contract with FDA allow up to 16 mg/100 kcal, the level in human milk.

Nucleotide supplementation of infant formula has been shown recently to enable infants to grow at a rate more similar to that of human milk-fed infants. Other studies have shown some benefits of supplemental nucleotides including the growth enhancement of babies born light for gestational age, fewer episodes of diarrhea, and improved immunological functions in babies' blood with greater antibody responses to diphtheria, oral polio virus, and *Hemophilus influenzae* immunizations.

Nucleotides are currently permitted to be added to conventional infant formulas.

Yeast-nonsynthetic is currently listed on the National List at §205.605(a). This designation includes Autolysate, Bakers, Brewers, and Nutritional Yeast. 5'-Monophosphate nucleotides produced by controlled hydrolysis of Brewers yeast RNA are a special form of yeast autolysate/yeast extract/nutritional yeast. Unless the hydrolysis of the yeast RNA is carefully controlled, the 5'-monophosphate nucleotides will not be produced. Thus, the current forms of yeast autolysates/extracts used for ordinary food flavoring uses are not acceptable in this infant formula application.

For these reasons, nucleotides isolated from Yeast RNA Hydrolysate should be included on the National List at §205.605 to permit the production and sale of "organic" infant formula fortified with nucleotides to the human milk level.

13. Confidential Business Information Statement.

This petition contains no Confidential Business Information.

Appendices

Petition for addition to the National List of the Nucleotides isolated from Yeast RNA Hydrolysate on the National List of Substances Allowed as Ingredients in or on Processed Products Labeled as "organic" or "made with organic (specified ingredients or food group(s))."

Appendix A – Food Chemicals Codex monographs

- 5'-Adenylic Acid
- 5'–Cytidylic Acid
- Disodium 5'-Guanylate
- Disodium 5'-Uridylate
- Disodium 5'-Inosinate

Appendix B –Product Labels

Appendix C – MSDS

- 5'-Adenylic Acid
- 5'–Cytidylic Acid
- Disodium 5'-Guanylate
- Disodium 5'-Uridylate
- Disodium 5'-Inosinate

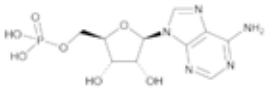
Appendix D – Nucleotide discussion – 1998 LSRO report (Pp. 2122S-2125S)

Monographs

Add the following:

▪5′-Adenylic Acid

Adenosine 5′-monophosphate
Adenylic acid
AMP
Adenosine 5′-phosphoric acid



$C_{10}H_{14}N_5O_7P$ Formula wt 347.23
CAS: [61-19-8]

DESCRIPTION

5′-Adenylic Acid occurs as colorless or white crystals, or as a white, crystalline powder. It is very slightly soluble in water, and practically insoluble in alcohol. It is produced by enzymatic cleavage of yeast ribonucleic acid (RNA) with a 5′-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of 5′-Adenylic Acid

Packaging and Storage: Store in tight containers protected from light and moisture.

IDENTIFICATION

- **A. Infrared Absorption**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP 5′-Adenylic Acid RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.
- **B. Procedure**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **Procedure**
  Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4) to a pH of 5.6
  Standard solution: 0.02 mg/mL of USP 5′-Adenylic Acid RS in *Mobile phase*. [Note—Ultra-sonication for 15 min at 30° may be necessary to aid in complete dissolution.]
  Sample solution: 0.02 mg/mL in *Mobile phase*. [Note—Ultra-sonication for 15 min at 30° may be necessary to aid in complete dissolution.]
  Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: UV 254 nm
  Column: 25 cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]
  Column temperature: Ambient
  Flow rate: About 1.0 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Suitability requirement 1: The relative standard deviation of the 5′-adenylic acid area responses from replicate injections is NMT 2.0%.
  Suitability requirement 2: The resolution, *R*, between the 5′-adenylic acid peak and all other peaks is NLT 2.0.
  Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for 5′-adenylic acid is 27.5 min.] Calculate the percentage of 5′-adenylic acid, $C_{10}H_{14}N_5O_7P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for 5′-adenylic acid in the *Sample solution*
r_S = peak area response for 5′-adenylic acid in the *Standard solution*
C_S = concentration of 5′-adenylic acid in the *Standard solution* (mg/mL)
C_U = concentration of the sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **Arsenic**
  [Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]
  Diluent: 4% nitric acid in water
  Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg /kg arsenic ICP standard solution
  Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic, from the *Standard stock solution* diluted with *Diluent*
  Sample: 5 g
  Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)
Setup: Use a suitable ICP–OES configured in a radial optical alignment. [NOTE—This method was developed using a Varian Vista MPX ICP–OES unit.] The instrument parameters are as follows: Set the ultra-violet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver the sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions*. [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of the *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL
Acceptance criteria: NMT 2 mg/kg

- **CADMIUM**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]
Diluent: 4% nitric acid in water
Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg/kg cadmium ICP standard solution
Standard solutions: 0.005, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium, from the *Standard stock solution* diluted with *Diluent*
Sample: 5 g
Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: ICP–OES
Setup: Same as that described in the test for *Arsenic*, but set to scan for cadmium at 228.802 nm
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of the *Sample* taken (g)
F =final volume of the *Sample solution*, 100 mL
Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]
Diluent: 4% nitric acid in water
Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution
Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead, from the *Standard stock solution* diluted with *Diluent*
Sample: 5 g
Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: ICP–OES
Setup: Same as that described in the test for *Arsenic*, but set to scan for lead at 220.353 nm
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of the *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL
Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]
Diluent: 4% nitric acid in water
Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg/kg mercury ICP standard solution

Standard solutions: 0.025, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury, from the *Standard stock solution* diluted with *Diluent*
Sample: 5 g
Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: ICP–OES
Setup: Same as that described in the test for *Arsenic,* but set to scan for mercury at 194.164 nm
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of the *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL
Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

• **ETHANOL**
Standard solution: 10 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Chromatographic system, Appendix IIA
Mode: Gas chromatography equipped with pressure-loop headspace autosampler
Detector: Flame ionization
Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]
Column temperature: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min
Injection port temperature: 140°
Detector temperature: 250°
Carrier gas: Nitrogen
Flow rate: 2.5 mL/min
Headspace unit: 2.5 mL/min
Equilibration temperature: 80°
Equilibration time: 60 min
Loop temperature: 85°
Transfer temperature: 90°
Pressurization time: 0.5 min
Loop fill time: 0.1 min
Injection time: 1 min
Injection size: 1 mL of headspace
System suitability
Sample: *Standard solution*
Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [NOTE—The approximate retention time for ethanol is 11 min.]
Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 100 mg/kg).

• **OTHER RIBONUCLEOTIDES**
Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.
Sample solution: 1.0 mg/mL. [NOTE—Ultra-sonication for 15 min at 30° may be necessary to aid in complete dissolution.]
Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS, each at 0.02 mg/mL in *Mobile phase*
Suitability requirements
Sample: *Standard solution*
Suitability requirement 1: The relative standard deviation of the 5′-adenylic acid peak area responses from replicate injections is NMT 2.0%.
Suitability requirement 2: The resolution, R, between the 5′-adenylic acid peak and all other nucleotide peaks is NLT 2.0.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from 5′-adenylic acid. [NOTE—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (5′-cytidylic acid, 5′-guanylic acid, 5′-inosinic acid, and 5′-uridylic acid) in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the analyte from the *Sample solution*
r_S = peak area of the analyte from the *Standard solution*
C_S = concentration of analyte in the *Standard solution* (mg/mL)
C_U = concentration of analyte in the *Sample solution* (mg/mL)
Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 0.5%, calculated on the anhydrous basis.

SPECIFIC TESTS

• **pH**, *pH Determination,* Appendix IIB
Sample solution: 0.05 mg/mL
Acceptance criteria: 3.3–4.3
• **WATER**, *Water Determination, Method I,* Appendix IIB
Acceptance criteria: NMT 6.0%

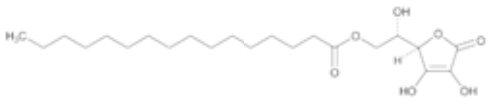
[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

- **BILE-TOLERANT GRAM-NEGATIVE BACTERIA,** Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **ENTEROBACTER SAKAZAKII** *(Cronobacter Spp.),* Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **SALMONELLA SPP.,** Appendix XIIC
  Sample preparation: Dissolve 25 g of the sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **TOTAL AEROBIC MICROBIAL COUNT,** *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 1,000 cfu/g
- **TOTAL YEASTS AND MOLDS COUNT,** *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 100 cfu/g■1S *(FCC7)*

Ascorbyl Palmitate

First Published: Prior to FCC 6

Palmitoyl L-Ascorbic Acid



$C_{22}H_{38}O_7$ Formula wt 414.54

INS: 304 CAS: [137-66-6]

DESCRIPTION

Ascorbyl Palmitate occurs as a white or yellow-white powder. It is very slightly soluble in water and in vegetable oils. One gram dissolves in about 4.5 mL of alcohol.

Function: Antioxidant

Packaging and Storage: Store in tightly closed containers, preferably in a cool, dry place.

IDENTIFICATION

Add the following:

- **■A. INFRARED ABSORPTION,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Ascorbyl Palmitate RS
  Sample and standard preparation: *K*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*■1S *(FCC7)*

Change to read:

- **■B.■1S *(FCC7)* PROCEDURE**
  Sample solution: 100 mg/mL in alcohol
  Acceptance criteria: The *Sample solution* decolorizes dichlorophenol–indophenol TS.

ASSAY

- **PROCEDURE**
  Sample: 300 mg
  Analysis: Dissolve the *Sample* in 50 mL of alcohol in a 250-mL Erlenmeyer flask. Add 30 mL of water and immediately titrate with 0.1 N iodine to a yellow color that persists for at least 30 s. Each mL of 0.1 N iodine is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.
  Acceptance criteria: NLT 95.0% of $C_{22}H_{38}O_7$, calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Flame Atomic Absorption Spectrophotometric Method,* Appendix IIIB
  Sample: 10 g
  Acceptance criteria: NMT 2 mg/kg

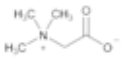
SPECIFIC TESTS

- **LOSS ON DRYING,** Appendix IIC**:** Vacuum oven at 56° to 60° for 1 h
  Acceptance criteria: NMT 2%
- **MELTING RANGE OR TEMPERATURE,** Appendix IIB
  Analysis: Determine as directed in *Procedure for Class Ia*
  Acceptance criteria: Between 107° and 117°
- **OPTICAL (SPECIFIC) ROTATION,** Appendix IIB
  Sample solution: 1 g in 10 mL of methanol
  Acceptance criteria: $[\alpha]_D^{25}$ between +21° and +24°, calculated on the dried basis
- **RESIDUE ON IGNITION (SULFATED ASH),** *Method I,* Appendix IIC
  Sample: 2 g
  Acceptance criteria: NMT 0.1%

Add the following:

■Betaine

Trimethylglycine
2-Trimethylammonioacetate
TMG
Glycine betaine
FEMA: 4223



$C_5H_{11}O_2N$ Formula wt, anhydrous 117.15
$C_5H_{11}O_2N \cdot H_2O$ Formula wt, monohydrate 135.16
CAS: anhydrous [107-43-7]
monohydrate [590-47-6]

DESCRIPTION

Betaine occurs as a white, very hygroscopic powder. It is recovered and purified from the aqueous liquor (molasses) remaining from the production of sucrose from sugar beets. It is very soluble in water, freely soluble in methanol, and soluble in ethanol.

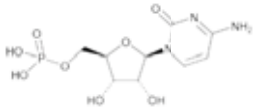
Function: Source of betaine, flavoring agent

Packaging and Storage: Store in well-closed containers.

Add the following:

▪5′-Cytidylic Acid

Cytidine 5′-monophosphate
Cytidylic acid
CMP
Cytidine 5′-phosphoric acid



$C_9H_{14}N_3O_8P$ Formula wt 323.20
CAS: [63-37-6]

DESCRIPTION

5′-Cytidylic Acid occurs as colorless or white crystals, or as a white, crystalline powder. It is very slightly soluble in water, and practically insoluble in alcohol. It is produced by enzymatic cleavage of yeast riboncleic acid (RNA) with a 5′-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of 5′-Cytidylic Acid

Packaging and Storage: Store in tight containers protected from light and moisture.

IDENTIFICATION

- **A. Infrared Absorption**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP 5′-Cytidylic Acid RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard*.
- **B. Procedure**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **Procedure**
  Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4) to a pH of 5.6
  Standard solution: 0.02 mg/mL of USP 5′-Cytidylic Acid RS in *Mobile phase*. [Note—Ultrasonication for 15 min at 30° may be necessary to aid in complete dissolution.]
  Sample solution: 0.02 mg/mL in *Mobile phase*. [Note—Ultrasonication for 15 min at 30° may be necessary to aid in complete dissolution.]
  Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: UV 254 nm
  Column: 25-cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]
  Column temperature: Ambient
  Flow rate: About 1.0 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Suitability requirement 1: The relative standard deviation of the 5′-cytidylic acid peak area responses from replicate injections is NMT 2.0%.
  Suitability requirement 2: The resolution, R, between the 5′-cytidylic acid peak and all other peaks is NLT 2.0.
  Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [Note—The approximate retention time for 5′-cytidylic acid is 4.6 min.] Calculate the percentage of disodium 5′-cytidylic acid, $C_9H_{14}N_3O_8P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response of 5′-cytidylic acid in the *Sample solution*
r_S = peak area response of 5′-cytidylic acid in the *Standard solution*
C_S = concentration of 5′-cytidylic acid in the *Standard solution* (mg/mL)
C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **Arsenic**
  [Note—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]
  Diluent: 4% nitric acid in water
  Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg /kg arsenic ICP standard solution
  Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic: from *Standard stock solution* diluted with *Diluent*
  Sample: 5 g
  Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
  Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
  Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)
  Setup: Use a suitable ICP–OES configured in a radial optical alignment. [Note—This method was developed using a Varian Vista MPX ICP OES unit.] The instrument parameters are as follows: set the ultraviolet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the

[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multi-channel peristaltic pump set to deliver sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions*. [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 2 mg/kg

- **CADMIUM**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg /kg cadmium ICP standard solution

Standard solutions: 0.005, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry*, Appendix IIC
Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)
Setup: Same as that described in the test for *Arsenic*, but set to scan for cadmium at 228.802 nm

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions*. [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry*, Appendix IIC
Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)
Setup: Same as that described in the test for *Arsenic*, but set to scan for lead at 220.353 nm

Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions*. [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[NOTE—When water is specified as a diluent, use deionized ultra-filtered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg /kg mercury ICP standard solution

Standard solutions: 0.025, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry*, Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)
Setup: Same as that described in the test for *Arsenic,* but set to scan for mercury at 194.164 nm
Analysis: Generate a standard curve using *Diluent* as a *Blank* and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = final volume of the *Sample solution*, 100 mL

Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

- **ETHANOL**

Standard solution: 20 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Chromatographic system, Appendix IIA
Mode: Gas chromatography equipped with pressure-loop headspace autosampler
Detector: Flame ionization
Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]
Temperature
Column: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min
Injection port: 140°
Detector: 250°
Carrier gas: Nitrogen
Flow rate: 2.5 mL/min
Headspace unit: 2.5 mL/min
Equilibration temperature: 80°
Equilibration time: 60 min
Loop temperature: 85°
Transfer temperature: 90°
Pressurization time: 0.5 min
Loop fill time: 0.1 min
Injection time: 1 min
Injection size: 1 mL of headspace
System suitability
Sample: *Standard solution*
Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [NOTE—The approximate retention time for ethanol is 11 min.]
Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 200 mg/kg).

- **OTHER RIBONUCLEOTIDES**

Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.
Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS each at 0.02 mg/mL in *Mobile phase*
Sample solution: 1.0 mg/mL. [NOTE—Ultrasonication for 15 min at 30° may be necessary to aid in complete dissolution.]
Suitability requirements
Sample: *Standard solution*
Suitability requirement 1: The relative standard deviation of the 5′-cytidylic acid peak area responses from replicate injections is NMT 2.0%.
Suitability requirement 2: The resolution, R, between the 5′-cytidylic acid peak and all other nucleotide peaks is NLT 2.0.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from 5′-cytidylic acid. [NOTE—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (disodium 5′-uridylate, 5′-guanylic acid, 5′-inosinic acid, and 5′-adenylic acid) in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the analyte from the *Sample solution*
r_S = peak area of the analyte from the *Standard solution*
C_S = concentration of analyte in the *Standard solution* (mg/mL)
C_U = concentration of analyte in the *Sample solution* (mg/mL)

Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 0.5%, calculated on the anhydrous basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB

Sample solution: 5 mg/mL
Acceptance criteria: 2.7–3.7

- **WATER**, *Water Determination, Method I,* Appendix IIB

Acceptance criteria: NMT 6.0%

- **BILE-TOLERANT GRAM-NEGATIVE BACTERIA**, Appendix XIIC

Sample preparation: Proceed as directed using a 10-g sample and incubating at 30°–35° for 18–24 h.
Acceptance criteria: Negative in 10 g

[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

- **ENTEROBACTER SAKAZAKII** *(Cronobacter spp.),* Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30°–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **SALMONELLA SPP.**, Appendix XIIC
  Sample preparation: Dissolve 25 g of sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **TOTAL AEROBIC MICROBIAL COUNT**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 1,000 cfu/g
- **TOTAL YEASTS AND MOLDS COUNT**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 100 cfu/g■1S (FCC7)

Add the following:

▪DHA from Algal (*Crypthecodinium*) Oil

Crypthecodinium cohnii Oil

DESCRIPTION

DHA from Algal (*Crypthecodinium*) Oil occurs as a light yellow to orange colored oil providing a source of docosahexaenoic acid (DHA, $C_{22}H_{32}O_2$) (C22:6 n-3), an omega-3 long-chain polyunsaturated fatty acid. It is obtained from fermentation of the species of microalgae *Crypthecodinium cohnii*, usually by solvent extraction. The oil may be winterized, bleached, and deodorized to substantially remove free fatty acids, phospholipids, odor and flavor components, and other material. Docosahexaenoic acid is the only significant polyunsaturated fatty acid present; DHA content may be standardized with other oils. Suitable antioxidants may be added.

Function: Source of DHA

Packaging and Storage: Store in tight, light-resistant containers. Avoid exposure to excessive heat.

IDENTIFICATION

- **FATTY ACID COMPOSITION,** *Fatty Acid Composition (Saturated,* cis-*Monounsaturated, and* cis-*Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII
  Acceptance criteria: The retention time of the peak of the docosahexaenoic acid methyl ester from the *Sample Preparation* corresponds to that from the *Standard Solution*. The area percentage for the methyl esters of the fatty acids from the *Sample Preparation* meet the requirements for each fatty acid indicated in the table below.

Fatty Acid	Shorthand Notation	Lower Limit (area %)	Upper Limit (area %)
Linoleic acid	18:2 n-6	0	1.0
Dihomo-gamma-linolenic acid	20:3 n-6	0	0.1
Eicosapentanoic acid	20:5 n-3	0	0.1
Docosapentaenoic acid	22:5 n-6	0	0.1
Docosahexaenoic acid	22:6 n-3	40.0	47.0

ASSAY

- **DHA,** *Fatty Acid Composition (Saturated,* cis-*Monounsaturated, and* cis-*Polyunsaturated) in Oils Containing Long Chain Polyunsaturated Fatty Acids,* Appendix VII
  Acceptance criteria: NLT 40.0% docosahexaenoic acid (DHA)

IMPURITIES

Inorganic Impurities

- **ARSENIC**

  Apparatus

  Sample digestion: Use a microwave oven (CEM Model MDS-2100, or equivalent) equipped with advanced composite vessels with 100-mL Teflon liners. Use rupture membranes to vent vessels should the pressure exceed 125 psi. The vessels fit into a turntable, and each vessel can be vented into an overflow container. Equip the microwave oven with an exhaust tube to ventilate fumes.

  Sample analysis: Use a suitable graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with an autosampler, pyrolytically coated graphite tubes, solid pyrolytic graphite platforms, and an adequate means of background correction. This method was developed using a Perkin-Elmer Model 5100, HGA-600 furnace, and an AS-60 autosampler with Zeeman effect background correction. An electrodeless discharge lamp serves as the source, argon as the purge gas, and air as the alternate gas. Set up the instrument according to manufacturer's specifications, with consideration of current good GFAAS practices. The instrument parameters are as follows:

  Wavelength: 193.7 nm
  Lamp current: 300 (EDL) modulated
  Pyrolysis: 1000°
  Atomization: 2400°
  Slit: 0.7
  Characteristic mass: 15 pg

  Glassware: Acid wash all glass, Teflon, and plastic vessels by soaking them in a nitric acid bath containing a 4:1 solution of water:nitric acid. [**CAUTION—**Wear a full face shield and protective clothing and gloves at all times when working with acid baths.] After acid soaking, rinse acid-washed items in deionized water, dry them, and store them in clean, covered cabinets.

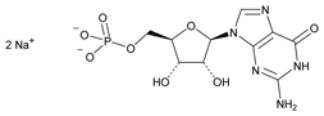
  Calibration standard stock solution: 100 µg/L

  Prepare from a suitable standard, which may be purchased (accuracy certified against National Institute of Standards and Technology [NIST] spectrometric standard solutions).

Disodium Guanylate

First Published: Prior to FCC 6

Disodium 5′-Guanylate
Disodium Guanosine-5′-monophosphate



$C_{10}H_{12}N_5Na_2O_8P{\cdot}xH_2O$ Formula wt 407.19

INS: 627 CAS: [5550-12-9]

DESCRIPTION

Disodium Guanylate occurs as colorless or white crystals, or as a white, crystalline powder. It contains approximately seven molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.

Function: Flavor enhancer

Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **Ultraviolet Absorption Spectrum**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Acceptance criteria: The ultraviolet absorption spectrum of the *Sample solution* exhibits an absorbance maximum at 256 ± 2 nm.

ASSAY

- **Procedure**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Standard solution: 20 µg/mL of USP Disodium Guanylate RS in 0.01 N hydrochloric acid
  Analysis: Using a suitable spectrophotometer set to the absorbance maximum at about 260 nm with 1-cm cells and 0.01 N hydrochloric acid as the blank, determine the absorbance of the *Sample solution* and of the *Standard solution*. Calculate the quantity, in mg, of $C_{10}H_{12}N_5Na_2O_8P$ in the sample taken by the formula:

$$\text{Result} = 25C \times A_U / A_S$$

C = exact concentration (µg/mL) of the *Standard solution*
A_U = absorbance of the *Sample solution*
A_S = absorbance of the *Standard solution*

  Acceptance criteria: NLT 97.0% and NMT 102.0% of $C_{10}H_{12}N_5Na_2O_8P$, calculated on the dried basis

IMPURITIES

Inorganic Impurities

- **Ammonium Salts**
  Sample: 100 mg
  Analysis: Transfer the *Sample* into a small test tube and add 50 mg of magnesium oxide and 1 mL of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min.
  Acceptance criteria: The litmus paper does not change to blue.
- **Lead,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Control: 5 µg Pb (5 mL of *Diluted Standard Lead Solution*)
  Acceptance criteria: NMT 5 mg/kg

Organic Impurities

- **Amino Acids**
  Sample solution: 1 mg/mL
  Analysis: Add 1 mL of ninhydrin TS to 5 mL of the *Sample solution* and heat for 3 min.
  Acceptance criteria: No color appears.
- **Other Nucleotides**
  Sample solution: 10 mg/mL
  Chromatographic system, Appendix IIA
  Mode: Descending chromatography (see *Paper Chromatography,* Appendix IIA)
  Stationary phase: Prepare a strip of Whatman No. 2, or equivalent, filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end.
  Solvent mixture: Saturated ammonium sulfate solution:*tert*-butyl alcohol:0.025 N ammonia (160:3:40)
  Application volume: 10 µL
  Detection/visualization: UV, 254 nm
  Analysis: Using a micropipette, apply the *Sample solution* to the center of the line drawn across the filter paper and dry in air. Fill the trough of an apparatus suitable for descending chromatography with the *Solvent mixture*. Suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front descends to a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark.
  Acceptance criteria: Only one spot is visible.

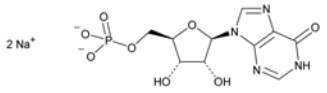
SPECIFIC TESTS

- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: 100 mg in 10 mL of water
  Acceptance criteria: The *Sample solution* is colorless and shows no more than a trace of turbidity.
- **LOSS ON DRYING,** Appendix IIC**:** 120° for 4 h
  Acceptance criteria: NMT 25.0%
- **pH,** *pH Determination,* Appendix IIB
  Sample solution: 50 mg/mL
  Acceptance criteria: Between 7.0 and 8.5

Disodium Inosinate

First Published: Prior to FCC 6

Disodium 5′-Inosinate
Disodium Inosine-5′-monophosphate



$C_{10}H_{11}N_4Na_2O_8P \cdot xH_2O$ Formula wt 392.17

INS: 631 CAS: [4691-65-0]

DESCRIPTION

Disodium Inosinate occurs as colorless or white crystals, or as a white, crystalline powder. It contains approximately 7.5 molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.
Function: Flavor enhancer
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **ULTRAVIOLET ABSORPTION SPECTRUM**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Acceptance criteria
  Absorbance maximum: 250 ± 2 nm
  A_{250}/A_{260} ratio: Between 1.55 and 1.65
  A_{280}/A_{260} ratio: Between 0.20 and 0.30

ASSAY

- **PROCEDURE**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Standard solution: 20 µg/mL of USP Disodium Inosinate RS in 0.01 N hydrochloric acid
  Analysis: Using a suitable spectrophotometer set to the absorbance maximum at about 250 nm with 1-cm cells and 0.01 N hydrochloric acid as the blank, determine the absorbance of the *Sample solution* and of the *Standard solution*. Calculate the quantity, in mg, of $C_{10}H_{11}N_4Na_2O_8P$ in the sample taken by the formula:

$$\text{Result} = 25C \times A_U / A_S$$

  C = exact concentration (µg/mL) of the *Standard solution*
  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  Acceptance criteria: NLT 97.0% and NMT 102.0% $C_{10}H_{11}N_4Na_2O_8P$, calculated on the anhydrous basis

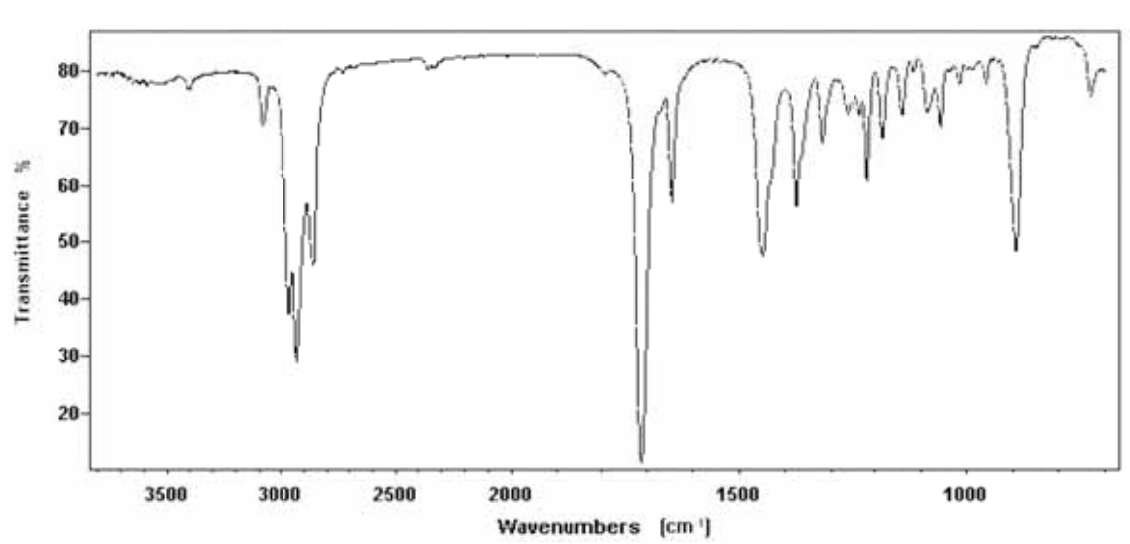
IMPURITIES

Inorganic Impurities

- **AMMONIUM SALTS**
  Sample: 100 mg
  Analysis: Transfer the *Sample* into a small test tube and add 50 mg of magnesium oxide and 1 mL of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min.
  Acceptance criteria: The litmus paper does not change to blue.
- **LEAD,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Control: 5 µg Pb (5 mL of *Diluted Standard Lead Solution*)
  Acceptance criteria: NMT 5 mg/kg

Organic Impurities

- **AMINO ACIDS**
  Sample solution: 1 mg/mL
  Analysis: Add 1 mL of ninhydrin TS to 5 mL of the *Sample solution*.
  Acceptance criteria: No color appears.



▪(+)-Dihydrocarvone▪1S *(FCC7)*

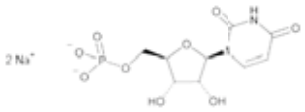
OTHER REQUIREMENTS

- **ANGULAR ROTATION**, *Optical (Specific) Rotation,* Appendix IIB
  Acceptance criteria: Between +14.0° and +22°

Add the following:

▪Disodium 5′-Uridylate

Uridine 5′-monophosphate disodium salt
Disodium uridine 5′-monophosphate
UMP disodium salt



$C_9H_{11}N_2Na_2O_9P \cdot xH_2O$ Formula wt 368.15
CAS: [3387-36-8]

DESCRIPTION

Disodium 5′-Uridylate occurs as colorless or white crystals. It contains approximately seven molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether. It is produced by enzymatic cleavage of yeast ribonucleic acid (RNA) with a 5′-phosphodiesterase followed by heat treatment, further purification steps, and washing of crystals with ethanol.

Function: Source of Disodium 5′-Uridylate

Packaging and Storage: Store in tight containers, protected from light and moisture.

IDENTIFICATION

- **A. INFRARED ABSORPTION**, *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Disodium 5′-Uridylate RS
  Sample and standard preparation: *A*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*
- **B. PROCEDURE**
  Acceptance criteria: The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Sample solution* corresponds to that of the *Standard solution* in the *Assay*.

ASSAY

- **PROCEDURE**
  Mobile phase: 0.1 M potassium dihydrogen phosphate (KH_2PO_4) in degassed water, adjusted to pH 5.6 with 0.1 M dipotassium hydrogen phosphate (K_2HP0_4)
  Standard solution: 0.02 mg/mL of USP Disodium 5′-Uridylate RS in *Mobile phase*. [NOTE—Ultrasonication may be necessary to aid in complete dissolution.]
  Sample solution: 0.02 mg/mL in *Mobile phase.* [NOTE—Ultrasonication may be necessary to aid in complete dissolution.]
  Chromatographic system, Appendix IIA
  Mode: High-performance liquid chromatography
  Detector: UV 254 nm
  Column: 25 cm × 4.6-mm; packed with 5-µm reversed phase C18 silica gel[1]
  Column temperature: Ambient
  Flow rate: About 1.0 mL/min
  Injection size: 50 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Suitability requirement 1: The relative standard deviation of the disodium 5′-uridylate peak area responses from replicate injections is NMT 2.0%.
  Suitability requirement 2: The resolution, R, between the disodium 5′-uridylate peak and all other peaks is NLT 2.0.

[1] YMC-Pack ODS-AQ (YMC Europe GmbH, Dinslaken, Germany), or equivalent.

Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for the major peaks on the resulting chromatograms. [NOTE—The approximate retention time for disodium 5′-uridylate is 6.2 min.] Calculate the percentage of disodium 5′-uridylate, $C_9H_{11}N_2Na_2O_9P$, in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area response for disodium 5′-uridylate in the *Sample solution*
r_S = peak area response for disodium 5′-uridylate in the *Standard solution*
C_S = concentration of disodium 5′-uridylate in the *Standard solution* (mg/mL)
C_U = concentration of sample in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–103.0%, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **ARSENIC**

[NOTE—When water is specified as a diluent, use deionized ultrafiltered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of arsenic as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of arsenic prepared by diluting a commercially available 1000 mg /kg arsenic ICP standard solution

Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of arsenic: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry*, Appendix IIC

Mode: Inductively coupled plasma–optical emission spectroscopy (ICP–OES)

Setup: Use a suitable ICP–OES configured in a radial optical alignment. [NOTE—This method was developed using a Varian Vista MPX ICP–OES unit.] The instrument parameters are as follows: set the ultraviolet detector to scan arsenic at 188.980 nm. Set the sample read time to 20 s. Set the forward power from the RF generator to 1150 watts. Use an argon plasma feed gas flow of 13.5 L/min with the auxiliary gas set to flow at 2.25 L/min. The sample is delivered to the spray chamber by a multichannel peristaltic pump set to deliver sample at a rate of 20 rpm. Samples are flushed through the system for 20 s prior to analysis. A 40-s read delay is also programmed into the sampling routine to allow for fluid flow equilibration after the high-speed flush, prior to the first analytical read of the sample. Between samples, the pumping system is washed by flushing the *Diluent* for 20 s.

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of arsenic in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of arsenic in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 2 mg/kg

- **CADMIUM**

[NOTE—When water is specified as a diluent, use deionized ultrafiltered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of cadmium as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of cadmium prepared by diluting a commercially available 1000 mg /kg cadmium ICP standard solution

Standard solutions: 0.005, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of cadmium: from *Standard stock solution* diluted with *Diluent*

Sample: 5 g

Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.

Spectrophotometric system, *Plasma Spectrochemistry*, Appendix IIC

Mode: ICP–OES

Setup: Same as that described in the test for *Arsenic*, but set to scan for cadmium at 228.802 nm

Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]

Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of cadmium in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of cadmium in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL

Acceptance criteria: NMT 0.1 mg/kg

- **LEAD**

[NOTE—When water is specified as a diluent, use deionized ultrafiltered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of lead as practical.]

Diluent: 4% nitric acid in water

Standard stock solution: 100 µg/mL of lead prepared by diluting a commercially available 1000 mg/kg lead ICP standard solution
Standard solutions: 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of lead: from *Standard stock solution* diluted with *Diluent*
Sample: 5 g
Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: ICP–OES
Setup: Same as that described in the test for *Arsenic*, but set to scan for lead at 220.353 nm
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of lead in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of lead in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL
Acceptance criteria: NMT 1 mg/kg

- **MERCURY**

[NOTE—When water is specified as a diluent, use deionized ultrafiltered water. When nitric acid is specified, use nitric acid of a grade suitable for trace element analysis with as low a content of mercury as practical.]
Diluent: 4% nitric acid in water
Standard stock solution: 100 µg/mL of mercury prepared by diluting a commercially available 1000 mg /kg mercury ICP standard solution
Standard solutions: 0.025, 0.05, 0.1, 0.2, 0.5, 1, and 2 µg/mL of mercury: from *Standard stock solution* diluted with *Diluent*
Sample: 5 g
Sample solution: Dissolve the *Sample* in 40 mL of 10% nitric acid in a 100-mL volumetric flask, and dilute with water to volume.
Spectrophotometric system, *Plasma Spectrochemistry,* Appendix IIC
Mode: ICP–OES
Setup: Same as that described in the test for *Arsenic*, but set to scan for mercury at 194.164 nm
Analysis: Generate a standard curve using *Diluent* as a blank and the *Standard solutions.* [NOTE—The correlation coefficient for the best-fit line should not be less than 0.999.]
Similarly, analyze the *Sample solution* on the ICP. Calculate the concentration (mg/kg) of mercury in the *Sample* taken:

$$\text{Result} = (C/W) \times F$$

C = concentration of mercury in the *Sample solution* determined from the standard curve (µg/mL)
W = weight of *Sample* taken (g)
F = *Sample solution* final volume, 100 mL
Acceptance criteria: NMT 0.5 mg/kg

Organic Impurities

- **ETHANOL**

Standard solution: 100 mg/kg of ethanol in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Sample solution: 100 mg/g in 1 N sodium hydroxide. Add 10 mL of this solution to a 20-mL headspace vial, and cap tightly.
Chromatographic system, Appendix IIA
Mode: Gas chromatography equipped with pressure-loop headspace autosampler
Detector: Flame ionization
Column: 30-m × 0.53-mm (id) capillary column with a 6% cyanopropylphenyl–94% dimethylpolysiloxane stationary phase and a 3.00-µm film thickness[2]
Temperature
Column: 20 min at 40°; increase to 240° at 10°/min; maintain at 240° for 10 min
Injection port: 140°
Detector: 250°
Carrier gas: Nitrogen
Flow rate: 2.5 mL/min
Headspace unit: 2.5 mL/min
Equilibration temperature: 80°
Equilibration time: 60 min
Loop temperature: 85°
Transfer temperature: 90°
Pressurization time: 0.5 min
Loop fill time: 0.1 min
Injection time: 1 min
Injection size: 1 mL of headspace
System suitability
Sample: *Standard solution*
Suitability requirement: The relative standard deviation of the ethanol peak area responses from replicate injections is NMT 5.0%.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, record the chromatograms, and measure the peak responses. [NOTE—The approximate retention time for ethanol is 11 min.]
Acceptance criteria: The peak area from the *Sample solution* does not exceed that from the *Standard solution* (NMT 1000 mg/kg).

- **OTHER RIBONUCLEOTIDES**

Mobile phase and **Chromatographic system:** Prepare as directed in the *Assay*.

[2] CP-Select 624 CB (Varian-Chrompack, Palo Alto, CA), or equivalent.

Sample solution: 1.0 mg/mL. [NOTE—Ultrasonication may be necessary to aid in complete dissolution.]
Standard solution: Mixture of USP Disodium 5′-Uridylate RS, USP 5′-Adenylic Acid RS, USP 5′-Cytidylic Acid RS, USP Disodium Guanylate RS, and USP Disodium Inosinate RS, each at 0.02 mg/mL in *Mobile phase*
Suitability requirements
Sample: *Standard solution*
Suitability requirement 1: The relative standard deviation of the disodium 5′-uridylate peak area responses from replicate injections is NMT 2.0%.
Suitability requirement 2: The resolution, R, between the disodium 5′-uridylate peak and all other nucleotide peaks is NLT 2.0.
Analysis: Separately inject equal volumes of the *Standard solution* and *Sample solution* into the chromatograph, and measure the responses for all nucleotide peaks on the resulting chromatograms, except the peak from disodium 5′-uridylate. [NOTE—The approximate retention times are 4.6 min (5′-cytidylic acid), 6.2 min (5′-uridylic acid), 10.3 min (5′-guanylic acid), 11.5 min (5′-inosinic acid), and 27.5 min (5′-adenylic acid).] Separately calculate the percentage of each analyte (5′-cytidylic acid, 5′-guanylic acid, 5′-inosinic acid, and 5′-adenylic acid) in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of the analyte from the *Sample solution*
r_S = peak area of the analyte from the *Standard solution*
C_S = concentration of analyte in the *Standard solution* (mg/mL)
C_U = concentration of analyte in the *Sample solution* (mg/mL)

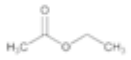
Acceptance criteria: The sum of the percentages for all nucleotide impurities is NMT 1%, calculated on the anhydrous basis.

SPECIFIC TESTS

- **pH**, *pH Determination,* Appendix IIB
  Sample solution: 50 mg/mL
  Acceptance criteria: 7.0–8.5
- **Water**, *Water Determination, Method I,* Appendix IIB
  Acceptance criteria: NMT 26.0%
- **Bile-Tolerant Gram-Negative Bacteria**, Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30°–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Enterobacter sakazakii** *(Cronobacter spp.),* Appendix XIIC
  Sample preparation: Proceed as directed using a 10-g sample and incubating at 30°–35° for 18–24 h.
  Acceptance criteria: Negative in 10 g
- **Salmonella spp.**, Appendix XIIC
  Sample preparation: Dissolve 25 g of sample at a sample/broth ratio of 1/8, and proceed as directed.
  Acceptance criteria: Negative in 25 g
- **Total Aerobic Microbial Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 1,000 cfu/g
- **Total Yeasts and Molds Count**, *Method I (Plate Count Method),* Appendix XIIB
  Acceptance criteria: NMT 100 cfu/g■1S (FCC7)

Ethyl Acetate

First Published: Prior to FCC 6
Last Revision: FCC 6



$C_4H_8O_2$ Formula wt 88.11
FEMA: 2414

DESCRIPTION

Ethyl Acetate occurs as a colorless liquid; volatile at low temperatures; flammable.
Odor: Acetous, ethereal
Solubility: Miscible in alcohol, ether, glycerin, most fixed oils, volatile oils; 1 mL dissolves in 10 mL water.

Change to read:

Boiling Point: ■~77°■1S (FCC7)
Function: Flavoring agent

IDENTIFICATION

- **Infrared Absorption,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Reference standard: USP Ethyl Acetate RS
  Sample and standard preparation: *F*
  Acceptance criteria: The spectrum of the sample exhibits maxima at the same wavelengths as those in the spectrum of the *Reference standard.*

ASSAY

- **Procedure:** Proceed as directed under *M-1b,* Appendix XI.
  Acceptance criteria: NLT 99.0% of $C_4H_8O_2$

SPECIFIC TESTS

- **Acid Value,** *M-15,* Appendix XI**:** Use bromocresol purple TS as the indicator.
  Acceptance criteria: NMT 5.0
- **Refractive Index,** Appendix II**:** At 20°
  Acceptance criteria: Between 1.370 and 1.375
- **Specific Gravity:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.894 and 0.898

OTHER REQUIREMENTS

- **Distillation Range,** Appendix IIB
  Acceptance criteria: Between 76° and 77.5°
- **Methyl Compounds,** *M-10,* Appendix XI
  Acceptance criteria: Passes test
- **Readily Carbonizable Substances,** *M-12,* Appendix XI
  Acceptance criteria: Passes test
- **Residue on Evaporation,** *M-16,* Appendix XI**:** 105°
  Sample: 10 g
  Acceptance criteria: NMT 0.02%

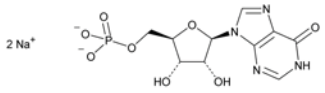
SPECIFIC TESTS

- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: 100 mg in 10 mL of water
  Acceptance criteria: The *Sample solution* is colorless and shows no more than a trace of turbidity.
- **LOSS ON DRYING,** Appendix IIC**:** 120° for 4 h
  Acceptance criteria: NMT 25.0%
- **pH,** *pH Determination,* Appendix IIB
  Sample solution: 50 mg/mL
  Acceptance criteria: Between 7.0 and 8.5

Disodium Inosinate

First Published: Prior to FCC 6

Disodium 5′-Inosinate
Disodium Inosine-5′-monophosphate



$C_{10}H_{11}N_4Na_2O_8P \cdot xH_2O$ Formula wt 392.17

INS: 631 CAS: [4691-65-0]

DESCRIPTION

Disodium Inosinate occurs as colorless or white crystals, or as a white, crystalline powder. It contains approximately 7.5 molecules of water of crystallization. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.
Function: Flavor enhancer
Packaging and Storage: Store in well-closed containers.

IDENTIFICATION

- **ULTRAVIOLET ABSORPTION SPECTRUM**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Acceptance criteria
  Absorbance maximum: 250 ± 2 nm
  A_{250}/A_{260} ratio: Between 1.55 and 1.65
  A_{280}/A_{260} ratio: Between 0.20 and 0.30

ASSAY

- **PROCEDURE**
  Sample solution: 20 µg/mL in 0.01 N hydrochloric acid
  Standard solution: 20 µg/mL of USP Disodium Inosinate RS in 0.01 N hydrochloric acid
  Analysis: Using a suitable spectrophotometer set to the absorbance maximum at about 250 nm with 1-cm cells and 0.01 N hydrochloric acid as the blank, determine the absorbance of the *Sample solution* and of the *Standard solution*. Calculate the quantity, in mg, of $C_{10}H_{11}N_4Na_2O_8P$ in the sample taken by the formula:

$$\text{Result} = 25C \times A_U / A_S$$

  C = exact concentration (µg/mL) of the *Standard solution*
  A_U = absorbance of the *Sample solution*
  A_S = absorbance of the *Standard solution*
  Acceptance criteria: NLT 97.0% and NMT 102.0% $C_{10}H_{11}N_4Na_2O_8P$, calculated on the anhydrous basis

IMPURITIES

Inorganic Impurities

- **AMMONIUM SALTS**
  Sample: 100 mg
  Analysis: Transfer the *Sample* into a small test tube and add 50 mg of magnesium oxide and 1 mL of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min.
  Acceptance criteria: The litmus paper does not change to blue.
- **LEAD,** *Lead Limit Test,* Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Control: 5 µg Pb (5 mL of *Diluted Standard Lead Solution*)
  Acceptance criteria: NMT 5 mg/kg

Organic Impurities

- **AMINO ACIDS**
  Sample solution: 1 mg/mL
  Analysis: Add 1 mL of ninhydrin TS to 5 mL of the *Sample solution*.
  Acceptance criteria: No color appears.

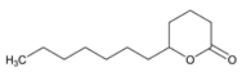
- **OTHER NUCLEOTIDES**
  Sample solution: 10 mg/mL
  Chromatographic system, Appendix IIA
  Mode: Descending chromatography (see *Paper Chromatography,* Appendix IIA)
  Stationary phase: Prepare a strip of Whatman No. 2, or equivalent, filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end.
  Solvent mixture: Saturated ammonium sulfate solution:*tert*-butyl alcohol:0.025 N ammonia (160:3:40)
  Application volume: 10 µL
  Detection/visualization: UV, 254 nm
  Analysis: Using a micropipette, apply the *Sample solution* to the center of the line drawn across the filter paper and dry in air. Fill the trough of an apparatus suitable for descending chromatography with the *Solvent mixture*. Suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front descends to a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark.
  Acceptance criteria: Only one spot is visible.

SPECIFIC TESTS

- **CLARITY AND COLOR OF SOLUTION**
  Sample solution: 500 mg in 10 mL of water
  Acceptance criteria: The *Sample solution* is colorless and shows no more than a trace of turbidity.
- **pH,** *pH Determination,* Appendix IIB
  Sample: 50 mg/mL
  Acceptance criteria: Between 7.0 and 8.5
- **WATER,** *Water Determination,* Appendix IIB
  Acceptance criteria: NMT 28.5%

δ-Dodecalactone

First Published: Prior to FCC 6



$C_{12}H_{22}O_2$ Formula wt 198.31
FEMA: 2401

DESCRIPTION

δ-Dodecalactone occurs as a colorless to yellow liquid.
Odor: Coconut-fruity, buttery on dilution
Solubility: Very soluble in alcohol, propylene glycol, vegetable oils; insoluble or practically insoluble in water
Boiling Point: ~140° to 141° (1 mm Hg)
Solubility in Alcohol, Appendix VI**:** One mL dissolves in 1 mL of 95% ethanol.
Function: Flavoring agent

IDENTIFICATION

- **INFRARED SPECTRA,** *Spectrophotometric Identification Tests,* Appendix IIIC
  Acceptance criteria: The spectrum of the sample exhibits relative maxima at the same wavelengths as those of the spectrum below.

ASSAY

- **PROCEDURE:** Proceed as directed under *M-1a,* Appendix XI.
  Acceptance criteria
  Sum of two isomers: NLT 98.0% of $C_{12}H_{22}O_2$
  δ Isomer: NLT 95.0% of $C_{12}H_{22}O_2$

SPECIFIC TESTS

- **ACID VALUE,** *Method II,* Appendix VII
  Acceptance criteria: NMT 8.0
- **REFRACTIVE INDEX,** Appendix II**:** At 20°
  Acceptance criteria: Between 1.458 and 1.461
- **SPECIFIC GRAVITY:** Determine at 25° by any reliable method (see *General Provisions*).
  Acceptance criteria: Between 0.942 and 0.950

OTHER REQUIREMENTS

- **SAPONIFICATION VALUE,** *Esters,* Appendix VI
  Sample: 1 g
  Acceptance criteria: Between 278 and 286

Similac® formulas for every baby's nutritional needs

Use as directed by your baby's doctor.

Advance	Sensitive	Expert Care
Complete nutrition for the 1st year	For babies with fussiness, gas, & spit-up	For babies needing extra TLC
Advance®	Sensitive	Alimentum®
Organic	Spit-Up	NeoSure®
	Soy	Diarrhea

And **Similac Go & Grow®** for toddlers learning to eat table food

Our **Feeding Expert hotline** is available to help you with feeding questions: **800-986-8800**

SAMPLE UPC 0 70074 59884 0

Breast milk is recommended. If you choose to use infant formula, the makers of Similac have a formula that's right for your baby.

Filled by volume to 1 Quart. Extra space in bottle allows for shaking and easy pouring.

Have product-related questions?
Call 1-800-515-7677,
8:30 am–5:00 pm,
Eastern time, weekdays.
www.Similac.com

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BIRTH TO 12 MONTHS
MILK-BASED

READY TO FEED — DO NOT ADD WATER

BRAND FED IN #1 HOSPITALS

Similac®
ADVANCE®

IMMUNE SUPPORT
Early Shield®
BRAIN & EYE
STRONG BONES
LUTEIN & DHA

USDA ORGANIC

Organic
Complete Nutrition

For Your Baby's 1st Year

Infant Formula with Iron

U-D 1 QT (946 mL)

NUTRIENTS PER 100 CALORIES (5 FL OZ)

PROTEIN	2.07	G	WATER	133	G
FAT	5.40	G	LINOLEIC ACID	860	MG
CARBOHYDRATE	10.9	G			

VITAMINS

VITAMIN A	300	IU	NIACIN	1050	MCG
VITAMIN D	60	IU	FOLIC ACID (FOLACIN)	15	MCG
VITAMIN E	1.5	IU	PANTOTHENIC ACID	450	MCG
VITAMIN K	8	MCG	BIOTIN	4.4	MCG
THIAMIN (VIT. B_1)	100	MCG	VITAMIN C (ASCORBIC ACID)	9	MG
RIBOFLAVIN (VIT. B_2)	150	MCG	CHOLINE	16	MG
VITAMIN B_6	60	MCG	INOSITOL	4.7	MG
VITAMIN B_{12}	0.25	MCG			

MINERALS

CALCIUM	78	MG	COPPER	90	MCG
PHOSPHORUS	42	MG	IODINE	6	MCG
MAGNESIUM	6	MG	SELENIUM	1.8	MCG
IRON	1.8	MG	SODIUM	24	MG
ZINC	0.75	MG	POTASSIUM	106	MG
MANGANESE	5	MCG	CHLORIDE	65	MG

INGREDIENTS: WATER, **ORGANIC** NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 0.5% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, CARRAGEENAN, MAGNESIUM CHLORIDE, SALT, FERROUS SULFATE, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, d-ALPHA-TOCOPHERYL ACETATE, L-CARNITINE, ZINC SULFATE, NIACINAMIDE, CALCIUM PANTOTHENATE, RIBOFLAVIN, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, POTASSIUM IODIDE, SODIUM SELENATE, VITAMIN D_3, CYANOCOBALAMIN, POTASSIUM HYDROXIDE AND NUCLEOTIDES (ADENOSINE 5'-MONOPHOSPHATE, CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE).
CONTAINS MILK AND SOY INGREDIENTS.
*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)

USE AS DIRECTED BY A DOCTOR
Directions for Preparation and Use

USE BY DATE ON BOTTLE • DO NOT ADD WATER
DO NOT USE IF BAND AROUND CAP OR INNER FOIL SEAL IS DAMAGED.

Your baby's health depends on carefully following these directions. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to boil (sterilize) bottles, nipples and rings before use.

Use

1. Shake very well before each use. Remove protective band; twist off and clean cap
2. Invert cap; press down to pierce foil, then turn cap a half turn. Remove foil
3. Pour formula into bottle; attach nipple. Once feeding begins, use within 1 hour or discard

Prepare and feed all formula in this container within 3 days after opening.

Storage Once opened, store quart bottle in refrigerator. Store prepared bottles in refrigerator and **feed to baby within 48 hours.** Store unopened containers at room temperature; avoid extreme temperatures. Do not reuse container.

Warning Do not use this container to warm formula. **Never use a microwave to warm formula.** Serious burns can result.

Abbott Nutrition, Abbott Laboratories, Columbus, Ohio 43219-3034 USA

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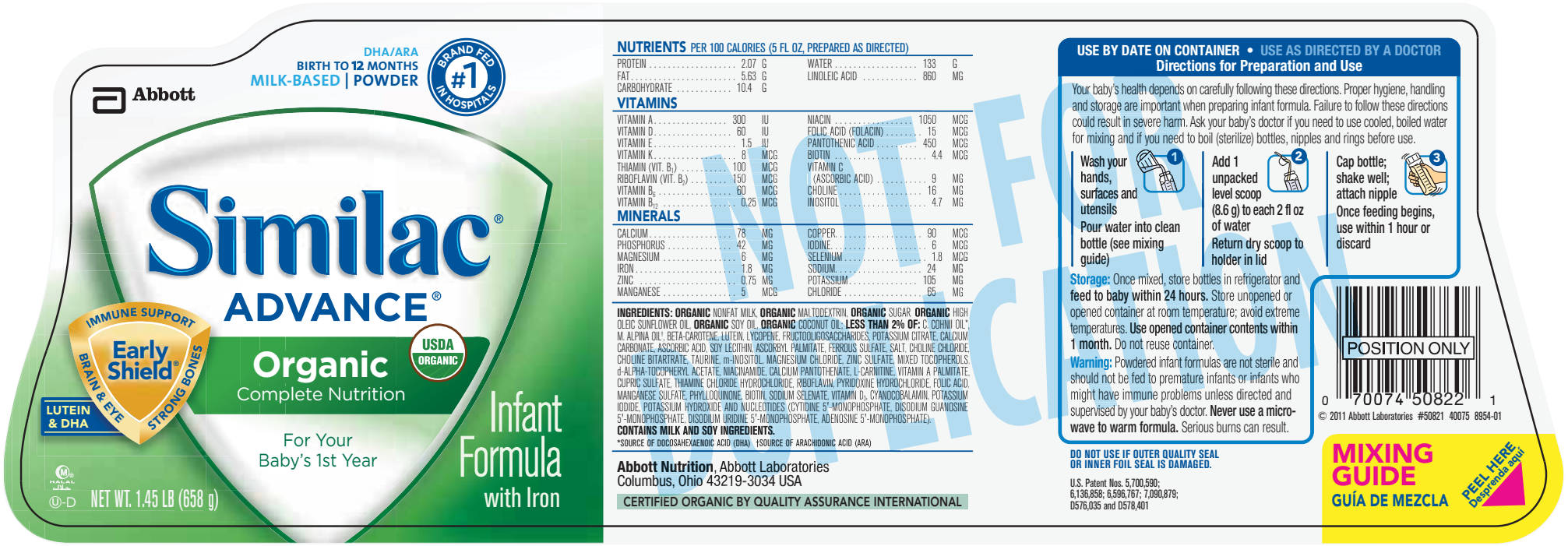

NUTRIENTS PER 100 CALORIES (5 FL OZ, PREPARED AS DIRECTED)

PROTEIN	2.07	G	WATER	133	G
FAT	5.63	G	LINOLEIC ACID	860	MG
CARBOHYDRATE	10.4	G			

VITAMINS

VITAMIN A	300	IU	NIACIN	1050	MCG
VITAMIN D	60	IU	FOLIC ACID (FOLACIN)	15	MCG
VITAMIN E	1.5	IU	PANTOTHENIC ACID	450	MCG
VITAMIN K	8	MCG	BIOTIN	4.4	MCG
THIAMIN (VIT. B_1)	100	MCG	VITAMIN C (ASCORBIC ACID)	9	MG
RIBOFLAVIN (VIT. B_2)	150	MCG	CHOLINE	16	MG
VITAMIN B_6	60	MCG	INOSITOL	4.7	MG
VITAMIN B_{12}	0.25	MCG			

MINERALS

CALCIUM	78	MG	COPPER	90	MCG
PHOSPHORUS	42	MG	IODINE	6	MCG
MAGNESIUM	6	MG	SELENIUM	1.8	MCG
IRON	1.8	MG	SODIUM	24	MG
ZINC	0.75	MG	POTASSIUM	105	MG
MANGANESE	5	MCG	CHLORIDE	65	MG

INGREDIENTS: ORGANIC NONFAT MILK, **ORGANIC** MALTODEXTRIN, **ORGANIC** SUGAR, **ORGANIC** HIGH OLEIC SUNFLOWER OIL, **ORGANIC** SOY OIL, **ORGANIC** COCONUT OIL; **LESS THAN 2% OF:** C. COHNII OIL*, M. ALPINA OIL†, BETA-CAROTENE, LUTEIN, LYCOPENE, FRUCTOOLIGOSACCHARIDES, POTASSIUM CITRATE, CALCIUM CARBONATE, ASCORBIC ACID, SOY LECITHIN, ASCORBYL PALMITATE, FERROUS SULFATE, SALT, CHOLINE CHLORIDE, CHOLINE BITARTRATE, TAURINE, m-INOSITOL, MAGNESIUM CHLORIDE, ZINC SULFATE, MIXED TOCOPHEROLS, d-ALPHA-TOCOPHERYL ACETATE, NIACINAMIDE, CALCIUM PANTOTHENATE, L-CARNITINE, VITAMIN A PALMITATE, CUPRIC SULFATE, THIAMINE CHLORIDE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, FOLIC ACID, MANGANESE SULFATE, PHYLLOQUINONE, BIOTIN, SODIUM SELENATE, VITAMIN D_3, CYANOCOBALAMIN, POTASSIUM IODIDE, POTASSIUM HYDROXIDE AND NUCLEOTIDES (CYTIDINE 5'-MONOPHOSPHATE, DISODIUM GUANOSINE 5'-MONOPHOSPHATE, DISODIUM URIDINE 5'-MONOPHOSPHATE, ADENOSINE 5'-MONOPHOSPHATE).
CONTAINS MILK AND SOY INGREDIENTS.
*SOURCE OF DOCOSAHEXAENOIC ACID (DHA) †SOURCE OF ARACHIDONIC ACID (ARA)

Abbott Nutrition, Abbott Laboratories
Columbus, Ohio 43219-3034 USA

CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL

USE BY DATE ON CONTAINER • USE AS DIRECTED BY A DOCTOR
Directions for Preparation and Use

Your baby's health depends on carefully following these directions. Proper hygiene, handling and storage are important when preparing infant formula. Failure to follow these directions could result in severe harm. Ask your baby's doctor if you need to use cooled, boiled water for mixing and if you need to boil (sterilize) bottles, nipples and rings before use.

1. Wash your hands, surfaces and utensils
   Pour water into clean bottle (see mixing guide)
2. Add 1 unpacked level scoop (8.6 g) to each 2 fl oz of water
   Return dry scoop to holder in lid
3. Cap bottle; shake well; attach nipple
   Once feeding begins, use within 1 hour or discard

Storage: Once mixed, store bottles in refrigerator and **feed to baby within 24 hours.** Store unopened or opened container at room temperature; avoid extreme temperatures. **Use opened container contents within 1 month.** Do not reuse container.

Warning: Powdered infant formulas are not sterile and should not be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor. **Never use a microwave to warm formula.** Serious burns can result.

DO NOT USE IF OUTER QUALITY SEAL OR INNER FOIL SEAL IS DAMAGED.

U.S. Patent Nos. 5,700,590; 6,136,858; 6,596,767; 7,090,879; D576,035 and D578,401

POSITION ONLY
0 70074 50822 1

MIXING GUIDE
GUÍA DE MEZCLA
PEEL HERE Despréndase aquí

IMPORTANT NOTICE: BREAST MILK IS BEST FOR BABIES. Before using an infant formula, ask the advice of your health-care professional. If you choose to supplement breastfeeding with formula or to formula feed exclusively, there is no better formula than Vermont Organics™ ORGANIC Infant Formula DHA and ARA. **Powdered infant formulas are NOT sterile and should NOT be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor.**

DIRECTIONS FOR PREPARATION AND USE

Your baby's health depends on carefully following these preparation, use and storage instructions; changes could affect your baby's nutrition and safety. Before preparing the infant formula, make sure to always wash your hands. Clean can top before opening. **Ask your baby's doctor about the need to boil or sterilize water for formula and the proper preparation of bottle and feeding utensils.**

1. Pour desired amount of warm water (approx. 100°F/40°C) into bottle. (See Feeding Chart below.)
2. Add powder. Always add powder to water.
3. Cap bottle and shake well until powder is dissolved. Feed immediately. Discard any remaining formula in bottle after 1 hour from start of feeding.

Use this Feeding Chart for proper amount of water and powder.

To Make	Water	Powder (Use scoop enclosed to measure)
2 fl oz bottle	2 fl oz	1 unpacked level **scoop** (8.7 g)
4 fl oz bottle	4 fl oz	2 unpacked level **scoops** (17.4 g)
6 fl oz bottle	6 fl oz	3 unpacked level **scoops** (26.1 g)
8 fl oz bottle	8 fl oz	4 unpacked level **scoops** (34.8 g)
1 quart	29 fl oz	1 unpacked level standard measuring **cup** & 2 unpacked level **scoops** (123 g)

Storage: Store unused prepared bottled formula in the refrigerator at 35-40°F (2-4°C); **use within 24 hours.** DO NOT FREEZE. Warm infant formula to room temperature and shake well before feeding. Prepared formula should not be without refrigeration more than 2 hours. Store open and unopened cans in a dry area at room temperature. Cover the opened can tightly with plastic cap; use contents within 1 month. Avoid any extreme temperatures.
Warning: Do not use microwave to prepare or warm formula. Serious burns may occur.
Filled by weight, not by volume. Contents may settle during shipment.
Contents yield approximately 190 fl oz of formula

Las instrucciones en Español se encuentran al dorso de esta etiqueta

Easy to Digest
Powder • Add Water

Vermont Organics™

For Babies
0-12 Months

organic infant formula with iron

Milk-Based Nutrition

Lipids – DHA & ARA
Nutrients found naturally in mother's milk

- No Antibiotics
- No Added Growth Hormones
- No Potentially Harmful Pesticides

USDA ORGANIC

NET WT 25.75 OZ (1 LB 9.75 OZ) 730 g

INGREDIENTS: **ORGANIC** REDUCED MINERALS WHEY, **ORGANIC** VEGETABLE OILS (PALM OR PALM OLEIN, HIGH OLEIC (SAFFLOWER OR SUNFLOWER), COCONUT, SOY), **ORGANIC** NONFAT MILK, **ORGANIC** LACTOSE, **AND LESS THAN 1%:** MORTIERELLA ALPINA OIL*, CRYPTHECODINIUM COHNII OIL**, VITAMIN A PALMITATE, BETA-CAROTENE, VITAMIN D (CHOLECALCIFEROL), VITAMIN E (dl-ALPHA TOCOPHERYL ACETATE), MIXED TOCOPHEROL CONCENTRATE, VITAMIN K (PHYTONADIONE), ASCORBYL PALMITATE, THIAMINE HYDROCHLORIDE, RIBOFLAVIN, PYRIDOXINE HYDROCHLORIDE, CYANOCOBALAMIN, NIACINAMIDE, FOLIC ACID, CALCIUM PANTOTHENATE, BIOTIN, ASCORBIC ACID, CHOLINE CHLORIDE, INOSITOL, CALCIUM CHLORIDE, CALCIUM HYDROXIDE, FERROUS SULFATE, ZINC SULFATE, MANGANESE SULFATE, CUPRIC SULFATE, POTASSIUM BICARBONATE, POTASSIUM CHLORIDE, POTASSIUM IODIDE, POTASSIUM HYDROXIDE, SODIUM SELENITE, SODIUM CITRATE, TAURINE, SOY LECITHIN, NUCLEOTIDES (ADENOSINE-5'-MONOPHOSPHATE, CYTIDINE-5'-MONOPHOSPHATE, DISODIUM GUANOSINE-5'- MONOPHOSPHATE, DISODIUM INOSINE-5'-MONOPHOSPHATE, DISODIUM URIDINE-5'MONOPHOSPHATE).
CONTAINS MILK AND SOY INGREDIENTS.

Diluted: Each 5 fl oz (150 mL) contains 100 Calories

NUTRIENTS:	PER 100 CALORIES:
PROTEIN g	2.2
FAT g	5.3
CARBOHYDRATE g	10.6
WATER g	134
LINOLEIC ACID mg	750
VITAMINS:	
VITAMIN A IU	300
VITAMIN D IU	60
VITAMIN E IU	2
VITAMIN K mcg	8
THIAMINE (VITAMIN B1) mcg	100
RIBOFLAVIN (VITAMIN B2) mcg	150
VITAMIN B6 mcg	63
VITAMIN B12 mcg	0.2
NIACIN mcg	750
FOLIC ACID (FOLACIN) mcg	7.5
PANTOTHENIC ACID mcg	315

VITAMINS:	PER 100 CALORIES:
BIOTIN mcg	2.3
VITAMIN C (ASCORBIC ACID) mg	9
CHOLINE mg	15
INOSITOL mg	4.1
MINERALS:	
CALCIUM mg	63
PHOSPHORUS mg	42
MAGNESIUM mg	7
IRON mg	1.8
ZINC mg	0.75
MANGANESE mcg	15
COPPER mcg	71
IODINE mcg	9
SELENIUM mcg	2.1
SODIUM mg	23
POTASSIUM mg	84
CHLORIDE mg	56

DISTRIBUTED BY:
VERMONT ORGANICS INFANT FORMULA
147 IND. PARK RD, GEORGIA, VT 05468

CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL 02FTP

*A SOURCE OF ARACHIDONIC ACID (ARA)
**A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)

Questions or Comments:
1-800-538-7615
9:00 a.m. to 5:00 p.m. EST
Monday – Friday

Produced under an ISO-9001 Quality System.

All infant formulas sold in the U.S. are required to be manufactured in accordance with, and meet the nutritional requirements of, the Federal Food, Drug and Cosmetic Act for infant formula under the regulation of the U.S. Food and Drug Administration.

lifes DHA•ARA

lifes®DHA•ARA™ is a trademark of Martek Biosciences Corporation.

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USE BEFORE DATE ON BOTTOM OF CAN

Breast milk is best. But if you decide to supplement breastfeeding with formula or formula-feed exclusively, Vermont Organics™ ORGANIC Infant Formula is a wholesome choice for your baby.

Made in the heart of the Green Mountains, Vermont Organics™ ORGANIC meets all USDA organic certification requirements. In addition to containing sources of organic fat, protein and carbohydrate, Vermont Organics™ ORGANIC is produced without the use of antibiotics, added growth hormones or potentially harmful pesticides.

L19847734-0351

IMPORTANT NOTICE: BREAST MILK IS BEST FOR BABIES. Before using an infant formula, ask the advice of your health-care professional. This organic soy infant formula powder is free of lactose and cow's milk protein. It is intended to meet the nutritional needs of term infants and babies who are sensitive to lactose or those who do not consume milk protein or milk products. **Powdered infant formulas are NOT sterile and should NOT be fed to premature infants or infants who might have immune problems unless directed and supervised by your baby's doctor.**

DIRECTIONS FOR PREPARATION AND USE

Your baby's health depends on carefully following these preparation, use and storage instructions; changes could affect your baby's nutrition and safety. Before preparing the infant formula, make sure to always wash your hands. Clean can top before opening. **Ask your baby's doctor about the need to boil or sterilize water for formula and the proper preparation of bottle and feeding utensils.**

1. Pour desired amount of warm water (approx. 100°F/40°C) into bottle. (See Feeding Chart below.)
2. Add powder. Always add powder to water.
3. Cap bottle and shake well until powder is dissolved. Feed immediately. Discard any remaining formula in bottle after 1 hour from start of feeding.

Use this Feeding Chart for proper amount of water and powder.

To Make	Water	Powder (Use scoop enclosed to measure)
2 fl oz bottle	2 fl oz	1 unpacked level **scoop** (8.8 g)
4 fl oz bottle	4 fl oz	2 unpacked level **scoops** (17.6 g)
6 fl oz bottle	6 fl oz	3 unpacked level **scoops** (26.4 g)
8 fl oz bottle	8 fl oz	4 unpacked level **scoops** (35.2 g)
1 quart	29 fl oz	1 unpacked level standard measuring **cup** & 2 unpacked level **scoops** (125 g)

Storage: Store unused prepared bottled formula in the refrigerator at 35-40°F (2-4°C); **use within 24 hours.** DO NOT FREEZE. Warm infant formula to room temperature and shake well before feeding. Prepared formula should not be without refrigeration more than 2 hours. Store open and unopened cans in a dry area at room temperature. Cover the opened can tightly with plastic cap; use contents within 1 month. Avoid any extreme temperatures.

Warning: Do not use microwave to prepare or warm formula. Serious burns may occur.

Filled by weight, not by volume. Contents may settle during shipment.

Contents yield approximately 187 fl oz of formula

Martek DHA

Las instrucciones en Español se encuentran al dorso de esta etiqueta

Easy to Digest
Powder • Add Water

Vermont Organics™

For Babies
0-12 Months

soy organic infant formula

with iron

Soy-Based Nutrition

Lipids – DHA & ARA

Nutrients found naturally in mother's milk

- Milk-Free
- Lactose-Free
- No Potentially Harmful Pesticides

USDA ORGANIC

Pareve

NET WT 25.75 OZ (1 LB 9.75 OZ) 730 g

INGREDIENTS: **ORGANIC** CORN SYRUP SOLIDS, **ORGANIC** SOY PROTEIN, **ORGANIC** PALM OLEIN OR PALM OIL, **ORGANIC** COCONUT OIL, **ORGANIC** HIGH OLEIC (SAFFLOWER OR SUNFLOWER) OIL, **ORGANIC** SOY OIL, **AND LESS THAN 1%:** MORTIERELLA ALPINA OIL*, CRYPTHECODINIUM COHNII OIL**, ASCORBYL PALMITATE, L-CARNITINE, L-METHIONINE, MIXED TOCOPHEROL CONCENTRATE, SOY LECITHIN, TAURINE, CALCIUM CHLORIDE, CALCIUM PHOSPHATE, CUPRIC SULFATE, FERROUS SULFATE, MAGNESIUM CHLORIDE, MANGANESE SULFATE, POTASSIUM BICARBONATE, POTASSIUM CHLORIDE, POTASSIUM CITRATE, POTASSIUM HYDROXIDE, POTASSIUM IODIDE, SODIUM CITRATE, SODIUM SELENITE, ZINC SULFATE, ASCORBIC ACID, BETA-CAROTENE, BIOTIN, CALCIUM PANTOTHENATE, CHOLINE BITARTRATE, CYANOCOBALAMIN, FOLIC ACID, INOSITOL, NIACINAMIDE, PYRIDOXINE HYDROCHLORIDE, RIBOFLAVIN, THIAMINE HYDROCHLORIDE, VITAMIN A PALMITATE, VITAMIN D (CHOLECALCIFEROL), VITAMIN E (dl-ALPHA TOCOPHERYL ACETATE), VITAMIN K (PHYTONADIONE). **CONTAINS SOY INGREDIENTS.**

Diluted: Each 5 fl oz (150 mL) contains 100 Calories

NUTRIENTS: PER 100 CALORIES:		*VITAMINS:* PER 100 CALORIES:	
PROTEIN g	2.5	BIOTIN mcg	3
FAT g	5.3	VITAMIN C (ASCORBIC ACID) mg	12
CARBOHYDRATE g	10.6	CHOLINE mg	24
WATER g	134	INOSITOL mg	6
LINOLEIC ACID mg	750	**MINERALS:**	
VITAMINS:		CALCIUM mg	105
VITAMIN A IU	300	PHOSPHORUS mg	69
VITAMIN D IU	60	MAGNESIUM mg	11
VITAMIN E IU	2	IRON mg	1.8
VITAMIN K mcg	8	ZINC mg	1.2
THIAMINE (VITAMIN B_1) mcg	80	MANGANESE mcg	25
RIBOFLAVIN (VITAMIN B_2) mcg	90	COPPER mcg	75
VITAMIN B_6 mcg	60	IODINE mcg	15
VITAMIN B_{12} mcg	0.3	SELENIUM mcg	2.8
NIACIN mcg	1000	SODIUM mg	36
FOLIC ACID (FOLACIN) mcg	16	POTASSIUM mg	120
PANTOTHENIC ACID mcg	500	CHLORIDE mg	80

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CERTIFIED ORGANIC BY QUALITY ASSURANCE INTERNATIONAL 02FTP

* A SOURCE OF ARACHIDONIC ACID (ARA) ** A SOURCE OF DOCOSAHEXAENOIC ACID (DHA)

Ⓟ PAREVE Contains no dairy ingredients. Manufactured on dairy equipment.

Questions or Comments:
1-800-538-7615
9:00 a.m. to 5:00 p.m. EST
Monday – Friday

Produced under an ISO-9001 Quality System.

All infant formulas sold in the U.S. are required to be manufactured in accordance with, and meet the nutritional requirements of, the Federal Food, Drug and Cosmetic Act for infant formula under the regulation of the U.S. Food and Drug Administration.

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USE BEFORE DATE ON BOTTOM OF CAN

Breast milk is best. But if you decide to supplement breastfeeding with formula or formula-feed exclusively, and lactose-sensitivity is a concern, Vermont Organics™ Soy ORGANIC Infant Formula is a wholesome choice for your baby.

Made in the heart of the Green Mountains, Vermont Organics™ Soy ORGANIC meets all USDA organic certification requirements. A milk-free, lactose-free baby formula containing organic soy protein.

L18A0730-0351

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No.88 Life First Road,DD Port,Dalian,China 116620
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Tel: 0086-411-39248033 Fax: 0086-411-39248233

MATERIAL SAFETY DATA SHEET

Adenosine-5'-Monophosphate Free Acid (5'-AMP)

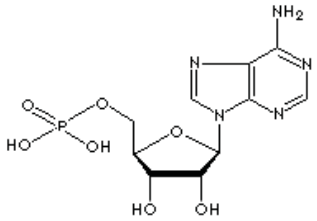
1. IDENTIFICATION OF THE SUBSTANCES/PREPARATION AND COMPANY

Product Name: Adenosine-5'-Monophosphate Free Acid (5'-AMP)

Nucleotide	CAS No.	Empirical Chemical Formula
Adenosine-5'-Monophosphate Free Acid (AMP)	61-19-8	$C_{10}H_{14}N_5O_7P$

Odor and taste: Odourless and characteristic taste.

Structures:



Product code:	03-002
Product type:	Nucleotides
Supplier/Manufacturer:	Dalian Zhen-Ao Bio-Tech Co.,Ltd. Subsidiary Company of ZHEN-AO GROUP CO., LTD.
Address:	No.88 Life First Road, DD Port, Dalian, China 116620
	Tel: +86-411-3924 8033 Fax: +86-411-3924 8233
EMERGENCY TELEPHONE:	**+86-411-3924 8033**

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients / Exposure Levels / LD50, Route, Species / LC50, Route, Species

—None.

3. HAZARDS INENTIFICATIOIN

Route of Entry:	None known
-Skin contact:	None known
-Skin absorption:	None known
-Eye contact:	None known
-Inhalation:	None known
-Ingestion:	Very Low toxicity
Effects of Acute Exposure:	No significant toxicity is expected. See above.
Effects of Chronic Exposure:	No chronic health effects are expected from normal use of this product.

4. FIRST AID MEASURES

Instructions: Immediately flush with large amounts of running water for at least 15 minutes.
Hold eyelids apart to ensure rinsing of the entire surface of the eye and lids

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with water. Flush contaminated skin with plenty of water.

Ingestion: No adverse effects anticipated by this route of exposure incidental to proper industrial handling. In case of inhalation, remove to fresh air. Consult a physician.

5. FIRE FIGHTING MEASURES

Flammability:	Not flammable
If yes, under which conditions?	
Extinguishing Media:	Water
Special Procedures:	No special instructions.
Flash Point ©, method:	N.AP.
Auto Ignition Temperature:	N.AV.
Upper Flammable Limit(%, by Vol.):	N.AP.
Lower Flammable Limit(%, by Vol.):	N.AP.
Explosion Data	
Explosive Power:	N.AV.
Rate of Burning:	N.AV.
Sensitivity of Static Discharge:	N. AV.
Sensitivity of Impact:	N.AV.
Unusual Fire and Explosion:	None
Hazards	
Hazardous Combustion Products:	None known.

6. ACCIDENTAL RELEASE MEASURES

Leaks/Spills: Use dry clean up procedures. Sweep or vacuum followed by water rinse. Keep dust to a minimum. Collect and contain in suitable disposal containers.

7. HANDLING AND STORAGE

Handing Procedures and Equipment:	Handle in accordance with good industrial hygiene and safety practices.
Storage Needs:	Store in a cool dry place. Store in a tightly closed container. Keep away from moisture, sunlight and open flame.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Gloves / Type:	Although this material does not present a significant skin concern, skin contamination should be minimized as good industrial practice.
Respiratory / Type:	None required under normal operating conditions. In dusty atmosphere, use an approved dust respirator.
Eye / Type:	Avoid eye contact as good industrial practice.
Footwear / Type:	No special requirements.
Clothing / Type:	No precautions other than clean body-covering clothing should be needed.
Other / Type:	Eye bath and safety shower.
Engineering Controls:	Ventilate adequately.

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9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	White powder or white crystalline powder.
Odour:	No odour
Odour Threshold:	N.AP.
Vapour Pressure (mmhg):	N.AV.
Vapour Density (Air=1):	N.AP.
Evaporation Rate:	N.AP.
Boiling Point:	N.AV.
Specific Gravity (Water=1):	N.AV.
Solubility in Water (%, w/w):	N.AV.
Coefficient of Water/Oil Dist:	N.AV.

10. STABILITY AND REACTIVITY

Chemical Stability: Yes.

Yes

No, which conditions?

Compatibility with other substances: Yes.

Yes

No, which ones?

Hazardous products of decomposition: None known

Hazardous polymerization: Will not occur.

11. TOXICOLOGICAL INFORMATION

Exposure Limit of Material:	N.AV.
LC 50 of Material, Species & Route:	N.AV.
LD 50 of Material, Species & Route:	N.AV.
Carcinogenicity of Material:	None
Reproductive Effects:	N.AV.
Irritancy of Material:	*See section 03.*
Sensitizing Capability of Material:	N.AV.
Synergistic Materials:	N.AV.

12. ECOLOGICAL INFORMATION

—None known

13. DISPOSAL CONSIDERATION

Waste Disposal: In accordance with National Regulations.

14. TRANSPORT INFORMATION

UN Number:	N.AP.
T.D.G. Classification:	Not Regulated.
Packing Group:	N.AP.
Special Shipping Instructions:	N.AP.

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15. REGULARTORY INFORMATION

FDA-USA：	These are approved as food additive used into Infant Formula.
EU：	These are approved as food additive used into Infant Formula under Commission Directive 2001/15/EC and Codex Standard For Infant Formula (Codex Stan 72-1981).
WHMIS Classification：	This is NOT a controlled product.

16. OTHER INFORMATION

N.AV. = Not Available
N.AP. = Not Applicable.

MSDS Main Reference:
Revision History: annual
Edition Number : ZAB-751-QD25-A/0
First issue date: 2009-01-15
Date of last revision: 2009-01-15
Disclaimer: The information contained in this date sheet is, to the best of our knowledge, true and accurate, but any recommendations or suggestions which may be made are without guarantee, since the conditions of use are beyond our control. Furthermore, nothing contained herein shall be construed as a recommendation to use any product in conflict with existing patents covering any material or its use.

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MATERIAL SAFETY DATA SHEET

Cytidine-5'-Monophosphate Free Acid (5'-CMP)

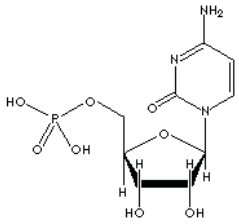
1. IDENTIFICATION OF THE SUBSTANCES/PREPARATION AND COMPANY

Product Name: Cytidine-5'-monophosphate Free Acid (5'-CMP)

Nucleotide	CAS No.	Empirical Chemical Formula
Cytidine-5'-Monophosphate Free Acid (CMP)	63-37-6	$C_9H_{14}N_3O_8P$

Odor and taste: Odourless and characteristic taste.

Structures:



Product code:	03-004
Product type:	Nucleotides
Supplier/Manufacturer:	Dalian Zhen-Ao Bio-Tech Co.,Ltd. Subsidiary Company of ZHEN-AO GROUP CO., LTD.
Address:	No.88 Life First Road, DD Port, Dalian, China 116620 Tel: +86-411-3924 8033 Fax: +86-411-3924 8233
EMERGENCY TELEPHONE:	**+86-411-3924 8033**

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients / Exposure Levels / LD50, Route, Species / LC50, Route, Species

—None.

3. HAZARDS INENTIFICATIOIN

Route of Entry:	None known
-Skin contact:	None known
-Skin absorption:	None known
-Eye contact:	None known
-Inhalation:	None known
-Ingestion:	Very Low toxicity
Effects of Acute Exposure:	No significant toxicity is expected. See above.
Effects of Chronic Exposure:	No chronic health effects are expected from normal use of this product.

4. FIRST AID MEASURES

Instructions: Immediately flush with large amounts of running water for at least 15 minutes. Hold eyelids apart to ensure rinsing of the entire surface of the eye and lids with water. Flush contaminated skin with plenty of water.

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Ingestion: No adverse effects anticipated by this route of exposure incidental to proper industrial handling. In case of inhalation, remove to fresh air. Consult a physician.

5. FIRE FIGHTING MEASURES

Flammability:	Not flammable
If yes, under which conditions?	
Extinguishing Media:	Water
Special Procedures:	No special instructions.
Flash Point ©, method:	N.AP.
Auto Ignition Temperature:	N.AV.
Upper Flammable Limit(%, by Vol.):	N.AP.
Lower Flammable Limit(%, by Vol.):	N.AP.
Explosion Data	
Explosive Power:	N.AV.
Rate of Burning:	N.AV.
Sensitivity of Static Discharge:	N. AV.
Sensitivity of Impact:	N.AV.
Unusual Fire and Explosion:	None
Hazards	
Hazardous Combustion Products:	None known.

6. ACCIDENTAL RELEASE MEASURES

Leaks/Spills: Use dry clean up procedures. Sweep or vacuum followed by water rinse. Keep dust to a minimum. Collect and contain in suitable disposal containers.

7. HANDLING AND STORAGE

Handing Procedures and Equipment:	Handle in accordance with good industrial hygiene and safety practices.
Storage Needs:	Store in a cool dry place. Store in a tightly closed container. Keep away from moisture, sunlight and open flame.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Gloves / Type:	Although this material does not present a significant skin concern, skin contamination should be minimized as good industrial practice.
Respiratory / Type:	None required under normal operating conditions. In dusty atmosphere, use an approved dust respirator.
Eye / Type:	Avoid eye contact as good industrial practice.
Footwear / Type:	No special requirements.
Clothing / Type:	No precautions other than clean body-covering clothing should be needed.
Other / Type:	Eye bath and safety shower.
Engineering Controls:	Ventilate adequately.

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9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	White powder or white crystalline powder.
Odour:	No odour
Odour Threshold:	N.AP.
Vapour Pressure (mmhg):	N.AV.
Vapour Density (Air=1):	N.AP.
Evaporation Rate:	N.AP.
Boiling Point:	N.AV.
Specific Gravity (Water=1):	N.AV.
Solubility in Water (%, w/w):	N.AV.
Coefficient of Water/Oil Dist:	N.AV.

10. STABILITY AND REACTIVITY

Chemical Stability:	Yes.
Yes	
No, which conditions?	
Compatibility with other substances:	Yes.
Yes	
No, which ones?	
Hazardous products of decomposition:	None known
Hazardous polymerization:	Will not occur.

11. TOXICOLOGICAL INFORMATION

Exposure Limit of Material:	N.AV.
LC 50 of Material, Species & Route:	N.AV.
LD 50 of Material, Species & Route:	N.AV.
Carcinogenicity of Material:	None
Reproductive Effects:	N.AV.
Irritancy of Material:	*See section 03.*
Sensitizing Capability of Material:	N.AV.
Synergistic Materials:	N.AV.

12. ECOLOGICAL INFORMATION

—None known

13. DISPOSAL CONSIDERATION

Waste Disposal: In accordance with National Regulations.

14. TRANSPORT INFORMATION

UN Number:	N.AP.
T.D.G. Classification:	Not Regulated.
Packing Group:	N.AP.
Special Shipping Instructions:	N.AP.

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15. REGULARTORY INFORMATION

FDA-USA：	These are approved as food additive used into Infant Formula.
EU：	These are approved as food additive used into Infant Formula under Commission Directive 2001/15/EC and Codex Standard For Infant Formula (Codex Stan 72-1981).
WHMIS Classification：	This is NOT a controlled product.

16. OTHER INFORMATION

N.AV. = Not Available
N.AP. = Not Applicable.

MSDS Main Reference:
Revision History: annual
Edition Number : ZAB-751-QD24-A/0
First issue date: 2009-01-15
Date of last revision: 2009-01-15
Disclaimer: The information contained in this date sheet is, to the best of our knowledge, true and accurate, but any recommendations or suggestions which may be made are without guarantee, since the conditions of use are beyond our control. Furthermore, nothing contained herein shall be construed as a recommendation to use any product in conflict with existing patents covering any material or its use.

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MATERIAL SAFETY DATA SHEET

Guanosine-5'-monophosphate Disodium Salt (5'-GMP, 2Na)

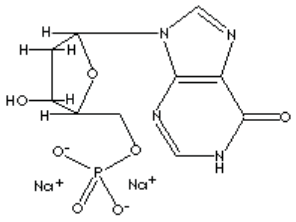
1. IDENTIFICATION OF THE SUBSTANCES/PREPARATION AND COMPANY

Product Name: Guanosine-5'-monophosphate Disodium Salt (5'-GMP, 2Na)

Nucleotide	CAS No.	Empirical Chemical Formula
Guanosine-5'-monophosphate Disodium Salt (GMP)	5550-12-9	$C_{10}H_{12}N_5Na_2O_8P \cdot 7.0H_2O$

Odor and taste: Odourless and characteristic taste.

Structures:



Product code: 03-005

Product type: Nucleotides

Supplier/Manufacturer: Dalian Zhen-Ao Bio-Tech Co.,Ltd.
Subsidiary Company of ZHEN-AO GROUP CO., LTD.

Address: No.88 Life First Road, DD Port, Dalian, China 116620
Tel: +86-411-3924 8033 Fax: +86-411-3924 8233

EMERGENCY TELEPHONE: +86-411-3924 8033

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients / Exposure Levels / LD50, Route, Species / LC50, Route, Species
—None.

3. HAZARDS INENTIFICATIOIN

Route of Entry: None known
-Skin contact: None known
-Skin absorption: None known
-Eye contact: None known
-Inhalation: None known
-Ingestion: Very Low toxicity
Effects of Acute Exposure: No significant toxicity is expected. See above.
Effects of Chronic Exposure: No chronic health effects are expected from normal use of this product.

4. FIRST AID MEASURES

Instructions: Immediately flush with large amounts of running water for at least 15 minutes. Hold eyelids apart to ensure rinsing of the entire surface of the eye and lids with water. Flush contaminated skin with plenty of water.

ZHEN-AO GROUP
ZHEN-AO GROUP CO., LTD.
No.88 Life First Road,DD Port,Dalian,China 116620
www.zhen-ao.com Email: trade@zhen-ao.com
Tel: 0086-411-39248033 Fax: 0086-411-39248233

Ingestion: No adverse effects anticipated by this route of exposure incidental to proper industrial handling. In case of inhalation, remove to fresh air. Consult a physician.

5. FIRE FIGHTING MEASURES

Flammability: Not flammable

If yes, under which conditions?

Extinguishing Media:	Water
Special Procedures:	No special instructions.
Flash Point ©, method:	N.AP.
Auto Ignition Temperature:	N.AV.
Upper Flammable Limit(%, by Vol.):	N.AP.
Lower Flammable Limit(%, by Vol.):	N.AP.

Explosion Data

Explosive Power:	N.AV.
Rate of Burning:	N.AV.
Sensitivity of Static Discharge:	N. AV.
Sensitivity of Impact:	N.AV.
Unusual Fire and Explosion:	None

Hazards

Hazardous Combustion Products:	None known.

6. ACCIDENTAL RELEASE MEASURES

Leaks/Spills: Use dry clean up procedures. Sweep or vacuum followed by water rinse. Keep dust to a minimum. Collect and contain in suitable disposal containers.

7. HANDLING AND STORAGE

Handing Procedures and Equipment: Handle in accordance with good industrial hygiene and safety practices.

Storage Needs: Store in a cool dry place. Store in a tightly closed container. Keep away from moisture, sunlight and open flame.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Gloves / Type:	Although this material does not present a significant skin concern, skin contamination should be minimized as good industrial practice.
Respiratory / Type:	None required under normal operating conditions. In dusty atmosphere, use an approved dust respirator.
Eye / Type:	Avoid eye contact as good industrial practice.
Footwear / Type:	No special requirements.
Clothing / Type:	No precautions other than clean body-covering clothing should be needed.
Other / Type:	Eye bath and safety shower.
Engineering Controls:	Ventilate adequately.

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9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	White powder or white crystalline powder.
Odour:	No odour
Odour Threshold:	N.AP.
Vapour Pressure (mmhg):	N.AV.
Vapour Density (Air=1):	N.AP.
Evaporation Rate:	N.AP.
Boiling Point:	N.AV.
Specific Gravity (Water=1):	N.AV.
Solubility in Water (%, w/w):	N.AV.
Coefficient of Water/Oil Dist:	N.AV.

10. STABILITY AND REACTIVITY

Chemical Stability:	Yes.
Yes	
No, which conditions?	
Compatibility with other substances:	Yes.
Yes	
No, which ones?	
Hazardous products of decomposition:	None known
Hazardous polymerization:	Will not occur.

11. TOXICOLOGICAL INFORMATION

Exposure Limit of Material:	N.AV.
LC 50 of Material, Species & Route:	N.AV.
LD 50 of Material, Species & Route:	N.AV.
Carcinogenicity of Material:	None
Reproductive Effects:	N.AV.
Irritancy of Material:	*See section 03.*
Sensitizing Capability of Material:	N.AV.
Synergistic Materials:	N.AV.

12. ECOLOGICAL INFORMATION

—None known

13. DISPOSAL CONSIDERATION

Waste Disposal: In accordance with National Regulations.

14. TRANSPORT INFORMATION

UN Number:	N.AP.
T.D.G. Classification:	Not Regulated.
Packing Group:	N.AP.
Special Shipping Instructions:	N.AP.

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15. REGULARTORY INFORMATION

FDA-USA：	These are approved as food additive used into Infant Formula.
EU：	These are approved as food additive used into Infant Formula under Commission Directive 2001/15/EC and Codex Standard For Infant Formula (Codex Stan 72-1981).
WHMIS Classification：	This is NOT a controlled product.

16. OTHER INFORMATION

N.AV. = Not Available
N.AP. = Not Applicable.

MSDS Main Reference:
Revision History: annual
Edition Number : ZAB-751-QD27-A/0
First issue date: 2009-01-15
Date of last revision: 2009-01-15
Disclaimer: The information contained in this date sheet is, to the best of our knowledge, true and accurate, but any recommendations or suggestions which may be made are without guarantee, since the conditions of use are beyond our control. Furthermore, nothing contained herein shall be construed as a recommendation to use any product in conflict with existing patents covering any material or its use.

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MATERIAL SAFETY DATA SHEET

Uridine-5'-monophosphate disodium salt (5'-UMP, 2Na)

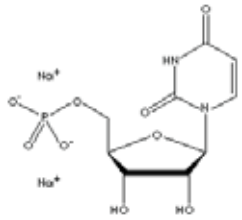
1. IDENTIFICATION OF THE SUBSTANCES/PREPARATION AND COMPANY

Product Name: Uridine-5'-monophosphate Disodium Salt (5'-UMP, 2Na)

Nucleotide	CAS No.	Empirical Chemical Formula
Uridine-5'-monophosphate Disodium Salt (UMP)	3387-36-8	$C_9H_{11}N_2O_9PNa_2$

Odor and taste: Odourless and characteristic taste.

Structures:



Product code:	03-003
Product type:	Nucleotides
Supplier/Manufacturer:	Dalian Zhen-Ao Bio-Tech Co.,Ltd. Subsidiary Company of ZHEN-AO GROUP CO., LTD.
Address:	No.88 Life First Road, DD Port, Dalian, China 116620 Tel: +86-411-3924 8033 Fax: +86-411-3924 8233
EMERGENCY TELEPHONE:	**+86-411-3924 8033**

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous Ingredients / Exposure Levels / LD50, Route, Species / LC50, Route, Species

—None.

3. HAZARDS INENTIFICATIOIN

Route of Entry:	None known
-Skin contact:	None known
-Skin absorption:	None known
-Eye contact:	None known
-Inhalation:	None known
-Ingestion:	Very Low toxicity
Effects of Acute Exposure:	No significant toxicity is expected. See above.
Effects of Chronic Exposure:	No chronic health effects are expected from normal use of this product.

4. FIRST AID MEASURES

Instructions: Immediately flush with large amounts of running water for at least 15 minutes. Hold eyelids apart to ensure rinsing of the entire surface of the eye and lids with water. Flush contaminated skin with plenty of water.

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Ingestion: No adverse effects anticipated by this route of exposure incidental to proper industrial handling. In case of inhalation, remove to fresh air. Consult a physician.

5. FIRE FIGHTING MEASURES

Flammability: Not flammable

If yes, under which conditions?

Extinguishing Media:	Water
Special Procedures:	No special instructions.
Flash Point ©, method:	N.AP.
Auto Ignition Temperature:	N.AV.
Upper Flammable Limit(%, by Vol.):	N.AP.
Lower Flammable Limit(%, by Vol.):	N.AP.

Explosion Data

Explosive Power:	N.AV.
Rate of Burning:	N.AV.
Sensitivity of Static Discharge:	N. AV.
Sensitivity of Impact:	N.AV.
Unusual Fire and Explosion:	None

Hazards

Hazardous Combustion Products:	None known.

6. ACCIDENTAL RELEASE MEASURES

Leaks/Spills: Use dry clean up procedures. Sweep or vacuum followed by water rinse. Keep dust to a minimum. Collect and contain in suitable disposal containers.

7. HANDLING AND STORAGE

Handing Procedures and Equipment: Handle in accordance with good industrial hygiene and safety practices.

Storage Needs: Store in a cool dry place. Store in a tightly closed container. Keep away from moisture, sunlight and open flame.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Gloves / Type:	Although this material does not present a significant skin concern, skin contamination should be minimized as good industrial practice.
Respiratory / Type:	None required under normal operating conditions. In dusty atmosphere, use an approved dust respirator.
Eye / Type:	Avoid eye contact as good industrial practice.
Footwear / Type:	No special requirements.
Clothing / Type:	No precautions other than clean body-covering clothing should be needed.
Other / Type:	Eye bath and safety shower.
Engineering Controls:	Ventilate adequately.

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9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	White powder or white crystalline powder.
Odour:	No odour
Odour Threshold:	N.AP.
Vapour Pressure (mmhg):	N.AV.
Vapour Density (Air=1):	N.AP.
Evaporation Rate:	N.AP.
Boiling Point:	N.AV.
Specific Gravity (Water=1):	N.AV.
Solubility in Water (%, w/w):	N.AV.
Coefficient of Water/Oil Dist:	N.AV.

10. STABILITY AND REACTIVITY

Chemical Stability:	Yes.
Yes	
No, which conditions?	
Compatibility with other substances:	Yes.
Yes	
No, which ones?	
Hazardous products of decomposition:	None known
Hazardous polymerization:	Will not occur.

11. TOXICOLOGICAL INFORMATION

Exposure Limit of Material:	N.AV.
LC 50 of Material, Species & Route:	N.AV.
LD 50 of Material, Species & Route:	N.AV.
Carcinogenicity of Material:	None
Reproductive Effects:	N.AV.
Irritancy of Material:	*See section 03.*
Sensitizing Capability of Material:	N.AV.
Synergistic Materials:	N.AV.

12. ECOLOGICAL INFORMATION

—None known

13. DISPOSAL CONSIDERATION

Waste Disposal: In accordance with National Regulations.

14. TRANSPORT INFORMATION

UN Number:	N.AP.
T.D.G. Classification:	Not Regulated.
Packing Group:	N.AP.
Special Shipping Instructions:	N.AP.

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15. REGULARTORY INFORMATION

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WHMIS Classification：	This is NOT a controlled product.

16. OTHER INFORMATION

N.AV. = Not Available
N.AP. = Not Applicable.

MSDS Main Reference:
Revision History: annual
Edition Number : ZAB-751-QD26-A/0
First issue date: 2009-01-15
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www.yamasa.com

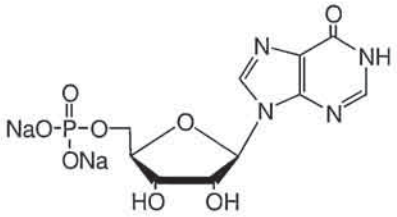
MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Trade name	: 5'-IMP-2Na
Chemical name	: Inosine 5'-monophosphate, disodium salt
CAS number	: 4691-65-0
Company name	: YAMASA CORPORATION
Address	: 10-1, Araoicho 2-Chome Choshi, Chiba-ken, 288-0056, JAPAN Telephone No. +81-479-22-0095

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous components : none
Structure :



33. HAZARDS IDENTIFICATION

Emergency overview : none
Potential health effects

Inhalation	: Causes respiratory tract irritation. May be harmful if inhaled.
Skin contact	: Causes skin irritation. May be harmful if absorbed through the skin.
Eye contact	: Causes eye irritation.
Ingestion	: May cause irritation of the digestive tract. May be harmful if swallowed.

4. FIRST AID MEASURES

Inhalation	: May be harmful, if a large amount of the material was taken, require immediately medical treatment.
Skin contact	: Wash immediately with water.
Eye contact	: Wash immediately with water.
Ingestion	: May be harmful, if a large amount of the material was taken, require immediately medical treatment.

5. FIRE FIGHTING MEASURES

Extinguishing media
Use extinguishing media appropriate to surrounding fire conditions.
Special firefighting procedures
Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Unusual fire and explosions hazards
Emits toxic fumes under fire conditions.

6. ACCIDENTAL RELEASE MEASURES

Wear self-contained breathing apparatus, rubber boots and heavy rubber gloves.
Sweep up, place in a bag and hold for waste disposal.
Avoid raising dust.
Ventilate area and wash spill site after material pickup is complete.

7. HANDLING AND STORAGE

Technical protective measures

Handling	: Do not breathe dust. Avoid contact with skin, eyes, or clothing. Wash thoroughly after handling.
Storage	: Keep dry, stable at room temperature.

8. EXPOSURE CONTROLS/ PERSONAL PROTECTION

Wear appropriate NIOSH / MSHA-approved respirator, chemical-resistant gloves, safety goggles, other protective clothing.

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Use only in a chemical fume hood.
Safety shower and eye bath.
Do not breathe vapor.
Do not get in eyes, on skin, on clothing.
Avoid prolonged or repeated exposure.
Wash thoroughly after handling.
Keep tightly closed.
Store in a cool day place.

9. PHYSICAL AND CHEMICAL PROPERTIES
Appearance and odor : white crystalline powder, odorless.
Formula : $C_{10}H_{11}N_4O_8PNa_2$
Molecular weight : 392.17
Vapor pressure : No data available.
Vapor density : No data available.
Solubility in water : Freely soluble in water.
Reactivity : Normally stable, not reactive with water.

10. STABILITY AND REACTIVITY
Stability : Stable under storage conditions.
Hazardous combustion or decomposition products :
Toxic fumes of: carbon monoxide, carbon dioxide, nitrogen oxides.
Hazardous polymerization : Will not occur.

11. TOXICOLOGICAL INFORMATION
Acute data (LD50(oral and dermal), irritation scores, LC50(inhalation)) :
No data available.

12. ECOLOGICAL INFORMATION
No data available.

13. DISPOSAL CONSIDERATIONS
Comply with all Federal, State, and local regulations.
Do not dump this material into sewers, on the ground or into any body of water.
Residue from fires extinguished with this material may be hazardous.
Do not re-use empty containers.

14. TRANSPORT INFORMATION
U.S. DOT hazardous material table(49 CFR 172.101) : Not listed.
Hazardous materials description/proper shipping name : None
Hazardous class : None
Identification number : None

15. REGULATORY INFORMATION
OSHA status : This product is not hazardous according to the OSHA Hazard Communication Standard, 29 CFR 1910.1200.
TSCA status (40 CFR 721): Reported (Included).
CERCLA reportable quantity (40 CFR 302): Not listed.
SARA title III
Section 302 (40 CFR 355) Appendix A and B : Not listed.
Section 311/312 (40 CFR 370) Hazard Categories :
Fire hazard : None., Sudden release : None., Reactive : None., Immediate (acute) health hazard : Not determined., Delay (chronic) health hazard :Not determined.
Section 313 (40 CFR 372) Toxic Chemicals : Not listed.

16. OTHER INFORMATIONS
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July 20, 2007 Version : 003

(Trachtman et al., 1988a,b). The attention given to taurine relative to its importance in pediatric nutrition is mostly attributable to its well-recognized role in fat digestion via its conjugation with bile acids to form bile salts, and its presumed role in the central nervous system based on data from animal studies (Sturman & Chesney, 1995).

Human milk concentrations of taurine have been reported in the range of 34 to 80 mg/L (5.1 to 11.9 mg/100 kcal)(Harzer et al., 1984; Rana & Sanders, 1986; Rassin et al., 1978), while bovine milk has very low taurine levels. In 1981, supplementation of term infant formula began in European communities, based on experimental evidence and clinical features of deficiency in patients who were nourished only with parenteral nutrition devoid of taurine (Sturman & Chesney, 1995). Since the FDA approval for supplementation in 1984, commercial infant formulas manufactured in the United States have been supplemented with taurine to compensate for the low amounts provided by bovine milk.

Review of extant data. Two lines of evidence support the essentiality of taurine in the diets of newborn infants: animal deficiency models and biochemical responses of infants (primarily preterm) provided taurine-free diets. The absence of taurine has been associated with the development of retinal degeneration in animal models including primates (Imaki et al., 1987; Neuringer & Sturman, 1987; Sturman et al., 1984). Sturman (1988) reported that the highest concentrations of taurine are found in the newborn and neonatal brain and are usually three- to four-times higher than in the mature brain. These data suggest that taurine may play an important role in the developmental process.

Most of the clinical studies involving taurine in humans have been performed in preterm infants (Sturman & Chesney, 1995). One exception was the study by Järvenpää et al. (1983), in which the amino acid profiles of plasma and urine were evaluated in term infants fed diets of taurine-free cow milk formula. Decreased levels of taurine were found in the plasma and urine of infants fed taurine-free formula.

Heird et al. (1987) reported that taurine supplementation during the administration of total parenteral nutrition may reduce the incidence and degree of cholestasis (impairment of bile secretion) in infants. Aside from this report, the Expert Panel was unable to find any additional studies conducted since 1985 which have evaluated the nutritional or toxicological aspects of taurine in term infants.

Conclusions and recommendations. **The Expert Panel found no compelling evidence to mandate the addition of taurine to formulas for term infants. However, the Expert Panel was aware of the history of use of taurine in formulas and the continued presence of taurine in some commercially available formulas. Consequently, the Expert Panel recommended a minimum taurine content of zero.**

The Expert Panel recommended a maximum taurine content of infant formulas of 12 mg/100 kcal, a value similar to the upper limit reported for human milk.

Nucleotides

Background. Nucleotides and their precursors are low-molecular-weight compounds that represent a small component of the nonprotein nitrogen portion of the human diet. The major nucleotides are the pyrimidine bases cytosine, thymine, and uracil, and the purine bases, adenine and guanine, to which a phosphorylated pentose sugar moiety is attached resulting in cytidine monophosphate (CMP), thymidine monophosphate (TMP), uridine monophosphate (UMP), adenine monophosphate (ADP) and guanosine monophosphate (GMP), respectively. Nucleosides are precursors to nucleotides and represent the pyrimidine or purine bases with the unphosphorylated pentose sugar only. The nucleotides function as precursors for the synthesis of the nucleic acids (ribonucleic acid;RNA and deoxyribonucleic acids; DNA) and are also fundamental to cell metabolism. Typically, nucleotides in mammalian cells are generated by *de novo* synthesis from amino acids or by the salvage pathway in which purine and pyrimidine products of protein catabolism are reutilized. These compounds exist in human milk as nucleosides, nucleotides, and nucleic acids. Human milk purine and pyrimidine bases are primarily in the form of nucleic acids.

The total free and cellular nucleotides content of human milk has been estimated to be as much as 20% of the nonprotein nitrogen (Uauy, 1989). According to values cited by Atkinson & Lönnerdal (1995), the RNA concentrations of human milk (100 to 5600 mg/L; 15 to 836 mg/100 kcal) are higher than those of DNA (10 to 120 mg/L; 1.5 to 18 mg/100 kcal). György (1971) observed that the nucleic acid levels of human milk are higher than those of cow milk. The source of nucleic acids in milk is unknown (Atkinson & Lönnerdal, 1995).

A wide range of values has been reported for each of the 13 nucleotide compounds that have been isolated from human milk. Uauy (1989) included a range of about 3 to 11 mg nucleotides/100 kcal in human milk (based on data of mean nucleotide concentrations of pooled milk samples collected 1 to 12 weeks postpartum). Carver & Walker (1995) summarizing data from nine reports, cited a range of 4 to 70 mg/L (0.59 to 10.4 mg/100 kcal) in their recent review of the literature on nucleotides. In two studies of human milk from European women, mean values of total

potentially available nucleosides (TPAN) of mature milk have been reported to be 10.1 to 16 mg/100 kcal (Leach et al., 1995; Thorell et al., 1996). The variation in values is primarily the result of lack of standardized collection and assay methodologies (Carver & Walker, 1995; Leach et al., 1995; Uauy, 1989).

The current CFR does not have minimum or maximum specifications for nucleotides (FDA, 1985a). Of the other authoritative agencies, only the Commission of the European Communities (1996), noted that "there is no justification for prohibiting their use..." in infant or follow-on formulas and set a maximum level of 5 mg/100 kcal (see Appendix A).

Review of extant data. Evidence exists to support the notion that dietary purines and pyrimidines may be semiessential nutrients (i.e., the endogenous supply is insufficient to maintain normal function), yet, the lack of nucleotides in the diet does not result in the development of an overt clinical deficiency syndrome (Uauy, 1989). One area for the investigation of a potential functional impact of nucleotide nutriture is the immune system. Possible roles for dietary nucleotides in the development and function of the immune system have been examined in animal models (Carver et al., 1991).

The hypothesized role of nucleotides in immune function is supported by studies in which the impact of congenital deficiency states of purine metabolism, such as adenosine deaminase deficiency and nucleoside phosphorylase deficiency, have been examined (Carson et al., 1977; Giblett et al., 1975). These enzyme deficiencies result in aberrant metabolism of nucleotides and impaired maturation and function of T and B lymphocytes, key components of the immune response. Additional immunologic effects attributed to nucleotides include macrophage activation, cytokine production and natural killer cell activity (Jyonouchi et al., 1993; Quan et al., 1990).

Other potential effects of dietary nucleotides including enhancement of iron absorption, enhancement of small intestinal development, changes in lipid metabolism, and influence on the intestinal microflora have been reviewed (Uauy, 1989). The hypothesized mechanism of enhancement of iron absorption is through the reduction and liberation of ferritin (releasing ferrous iron) by xanthine oxidase (Uauy, 1989). Faelli & Esposito (1970) conducted studies in rats and demonstrated that inosine, hypoxanthine, and uric acid increased iron absorption. In his review, Uauy (1989) noted increased iron absorption in breast-fed infants compared to infants fed formula. However, it is not possible to attribute this difference to nucleotides (versus other components of breast milk). No studies were found to corroborate a specific effect of nucleotides on iron absorption in humans.

Modification of intestinal microflora by nucleotides has been suggested by several clinical studies. Tanaka & Mutai (1980) reported that colonization by *Bifidobacterium* species, a microorganism normally found in the intestinal tract of breast-fed infants, could be promoted by the addition of nucleotides to infant formulas. Colonization of *Bifidobacterium* is desirable, as this species contributes to lowering the pH of the intestinal contents by hydrolyzing various sugars, thereby providing protection against the growth of pathogenic acid-intolerant strains of intestinal bacteria (Uauy, 1989).

Generally, studies of supplementation with nucleotides have involved varying proportions of the following nucleotides: adenosine-, guanosine-, cytidine-, uridine-, and inosine-5'-monophosphates. Gil et al. (1986) studied the effect of nucleotide supplementation (total nucleotide concentration approximately 11 mg/L: 1.6 mg/100 kcal) on fecal microbial flora of formula-fed infants. Infants were breast fed (n=10), or fed either conventional formula (n=12) or conventional formula supplemented with nucleotides (n=11). Fecal microbial flora was examined at one and four weeks. Gil et al. (1986) reported that the fecal *Bifidobacteria* and enteropathogenic bacterial patterns of the supplemented group were more similar to the breast-fed group than the group fed unsupplemented formula.

Balmer et al. (1994) compared fecal flora at two, four and seven weeks postpartum from healthy term breast-fed infants (mean total nucleotide concentration 11.8 mg/L or about 1.8 mg/100 kcal; n=21) and infants fed whey-based formula with (n= 32) or without supplemental nucleotides (34 mg total nucleotides/L; 5.1 mg/100 kcal; n=33). Despite the larger dose than used by Gil et al. (1986), no benefit was observed in the infants fed the supplemented formulas compared to the unsupplemented formula-fed infants. In fact, Balmer et al. (1994) reported that the supplemented infants not only did not have more *Bifidobacterium*, but there was a higher percentage of *Escherichia coli* particularly at two weeks. These authors concluded that nucleotide supplementation did not result in fecal flora analogous to that of breast-fed infants.

A large study was conducted in Chile to evaluate the effects of nucleotides on infant diarrheal disease (Brunser et al., 1994). In this double-blinded study, the incidence of diarrhea was evaluated in infants of lower socioeconomic populations who were fed conventional cow milk formula (n=148) or formula with added nucleotides (14.8 mg/L; 2.2 mg/100 kcal; n=141) over a period of three months. The supplemented group experienced fewer episodes of diarrhea (109 versus 140) than the conventional formula group; however, there were no differences in the clinical spectrum of the diarrheal episodes or in the

enteropathogens associated with the illness. Brunser et al. (1994) were unable to provide any insights into the potential mechanism(s) of the reported effects. Reasons for the large attrition rate (original group sizes were 194 and 198 for the supplemented and unsupplemented groups, respectively) included palatability of formulas, relocation, illness, and failure to participate in follow-up visits. The ability to generalize about the Brunser et al. (1994) findings is tempered by sample selection criteria, i.e., only subjects of lower socioeconomic status were studied.

Carver et al. (1991) evaluated natural killer cell cytotoxicity and interleukin-2 production of mononuclear cells collected at two and four months of age from breast-fed infants (n=9) and infants fed conventional formula (n=15) or conventional formula supplemented with nucleotides (33 mg total nucleotides/L; 5 mg/100 kcal; n=12). Natural killer cell activity and interleukin-2 levels were higher in the breast-fed and nucleotide-supplemented groups than in the infants fed conventional formula. No differences were reported between supplemented and breast-fed infants for any measure. No differences in growth or rates of infection were observed among the three groups.

Pickering et al. (1998) recently evaluated the impact of nucleotide supplementation of infant formula on the immune response to *Haemophilus influenzae* type b polysaccharide (Hib), diphtheria, and tetanus toxoids, and to oral polio virus (OPV) immunizations. A total of 311 full-term infants (out of 370 enrolled) completed the multisite study. The formula groups were randomized to receive either commercially available iron-fortified milk-based formula (n=107) or the same formula supplemented with 72 mg/L (10.7 mg/100 kcal) of nucleotides (n=101). A third group of infants who were exclusively breast-fed for at least two months and then fed breast milk or the unsupplemented conventional formula (n=103) was also studied. Antibody responses were determined at 6, 7 and 12 months of age. No differences in growth were observed among the three groups. The nucleotide-supplemented group had higher Hib and diphtheria antibody concentrations at seven months of age (one month after the sixth-month immunization). The higher Hib titer persisted at one year of age while the OPV and tetanus responses were not different among groups. The breast-fed group had a greater antibody response to OPV than either of the formula-fed groups. The nucleotide-supplemented group had a lower incidence of diarrhea during the first year than did the unsupplemented formula-fed group.

Another rationale used for the supplementation of infant formula with dietary nucleotides has been the potential beneficial effects on lipid metabolism. Sanchez-Pozo et al. (1986) measured the levels of plasma lipoproteins in breast-fed infants (n=26) and those fed commercial formula (n=35) or formula supplemented with nucleotides (n=23). The supplementation level was expressed as 8.6 mg/100 g dry formula. Presumably, the supplement was the same as reported for Gil et al. (1986), and was the equivalent of about 11 mg total nucleotides/L or 1.6 mg/100 kcal. At one month of age, plasma HDL levels were higher in the breast-fed and nucleotide-supplemented formula-fed infants than in the infants receiving conventional formula. VLDL levels were lower in the breast-fed and nucleotide-supplemented infants. No differences in growth rates were noted among the groups. This same group of investigators found similar effects from nucleotide supplementation in follow-up studies involving both term and preterm infants (Sanchez-Pozo et al., 1994, 1995). Although the mechanism(s) have not been elucidated, the authors suggested that nucleotides may regulate hepatic lipoprotein synthesis.

Two studies by the same research group investigated the impact of nucleotide supplementation on fatty acid concentrations in plasma (Gil et al., 1988) and erythrocytes (DeLucchi et al., 1987) of term infants. The study groups included breast-fed infants (n=20) and infants fed either nucleotide-supplemented (n=19) or unsupplemented (n=19) conventional formula. Blood samples were compared at 30 days of age. In both studies, nucleotide supplementation resulted in AA and DHA levels more closely resembling the fatty acid pattern observed in breast-fed infants than those of conventional formula-fed infants. The authors suggested that the augmentation of LCPUFAs was attributable to the impact of nucleotides on desaturation and elongation of essential fatty acids to LCPUFAs.

Conclusions and recommendations. **The Expert Panel found no compelling reason to require the addition of nucleotides to infant formulas at this time.** Preliminary evidence of beneficial effects from nucleotide supplementation of infant formulas is intriguing, and the Expert Panel strongly urges continued research in this area. When data from long-term, large-scale clinical trials are available, the question of addition of nucleotides to infant formulas should be reconsidered, preferably within five years.

The Expert Panel recommended a maximum content of nucleotides and nucleotide precursors in infant formula of 16 mg/100 kcal, a value similar to the upper limit reported for human milk. The Expert Panel specified that the maximum level of free nucleotides, including available nucleosides and nucleic acids (DNA and RNA) that serve as nucleotide precursors, be limited to the amount and composition present in human milk and not exceed 20% of the total nonprotein nitrogen supplied in

infant formula, and a maximum total level of 16 mg/100 kcal.

Glutathione

Background. Glutathione (γ-glutamylcysteinylglycine) is an endogenously produced tripeptide consisting of glutamic acid, cysteine and glycine. The sulfhydryl group of cysteine serves as the functional component by conjugating reactive electrophilic xenobiotics, such as methyl iodide and benzyl chloride (Sipes & Gandolfi, 1991). The conjugation reactions are catalyzed by glutathione S-transferase enzymes that are abundant in the cytosolic compartment of hepatocytes and in lesser quantities in other tissues such as the kidney, intestine and adrenal gland. In the kidney, the conjugated derivatives are cleaved to cysteine derivatives and subsequently excreted in the urine.

As a substrate for these conjugation reactions, glutathione serves a significant role in cellular defense mechanisms by preventing reactive electrophiles from interacting with DNA, RNA or cellular proteins. Glutathione also is required in red blood cells for the reduction of hydrogen peroxide and organic peroxides in a selenium-dependent reaction catalyzed by glutathione peroxidase (GPx) (discussed in Chapter VIII). In addition, glutathione serves a carrier for certain amino acids across plasma membranes, particularly in the kidney (Sipes & Gandolfi, 1991).

Glutathione is produced and degraded by a complex series of reactions termed the γ-glutamyl cycle. Other than hereditary deficiencies of the enzymes known to influence the synthesis and metabolism of glutathione, deficiency states have not been documented. Vitamin B_{12} appears to be important in the maintenance of the reduced form of glutathione. Deficiency states of vitamin B_{12} have been associated with decreased red blood cell and liver concentrations of reduced glutathione (Herbert & Das, 1994).

Although data exist on the content of glutathione peroxidase in human milk (Hamosh, 1995), no data were found on the glutathione content of human milk.

Currently, the FDA and other international authoritative organizations do not have minimum or maximum glutathione specifications for infant formula (see Appendix A).

Review of extant data. In the review of the literature, no studies were identified that specifically addressed the nutritional value of ingestion of glutathione. However, one enzyme for which glutathione is a substrate, glutathione peroxidase, is critically linked to dietary levels of selenium. A review of the nutritional issues relative to this enzyme can be found in the discussion on selenium in Chapter VIII.

The Expert Panel was unaware of any studies conducted to address the role of glutathione supplementation of formula for term infants.

Conclusions and recommendations. **The Expert Panel did not recommend the addition of glutathione to infant formulas.** There was no convincing evidence of a dietary requirement for glutathione.

Urea

The Expert Panel did not recommend the addition of urea to infant formulas. The urea content of human milk reflects the urea content of the lactating woman's blood, which, in turn, reflects recent dietary intake of nitrogen. In normal infants, the contribution of urea toward meeting protein needs appears to be low.

Glutamine

Background. Glutamine is the most abundant amino acid in the human body, accounting for about 20% of the free amino acids in plasma and ~60% of skeletal muscle. This amino acid serves as a substrate for ammonia production in the kidneys and functions in nitrogen transfer between tissues (Marliss et al., 1971). Other metabolic functions include serving as a substrate source for purine and pyrimidine biosynthesis, regulating protein synthesis and inhibiting protein degradation (MacLennan et al., 1987).

Under most circumstances, glutamine is synthesized in the amounts required for the infant and as such is nonessential in the diet. Some authors have argued that glutamine may be essential under conditions of physiologic stress (Lacey & Wilmore, 1990).

Neither the FDA nor other international authoritative agencies have glutamine specifications for infant formula (see Appendix A).

Review of extant data. Although investigations have been designed to address dietary supplementation of glutamine for very low-birthweight infants and for numerous other clinical populations, there have been no such studies conducted in healthy term infants. It should be noted, however, that glutamine is a constituent of many proteins contained in both human milk and infant formulas.

Conclusions and recommendations. **The Expert Panel did not recommend the addition of glutamine to infant formulas.** There was no convincing evidence of a dietary requirement for glutamine.

Glycine

Background. Glycine, a nonessential amino acid synthesized primarily from serine and threonine, participates in the biosynthesis of heme, purines, creatine, and glycine conjugates such as glycocholic acid and