International Formula Council

October 24, 2011

Lisa Brines, Ph.D.
National List Manager
USDA/AMS/NOP, Standards Division
1400 Independence Ave. SW
Room 2646-So., Ag Stop 0268
Washington, DC 20250-0268

RE: Petition for inclusion of L-Methionine on the National List at §205.605(b) as a synthetic non-agricultural substance allowed in or on processed infant formula products labeled as "organic" or "made with organic (specified ingredients)" with the annotation "for use only in infant formula based on isolated soy protein."

Dear Dr. Brines,

The International Formula Council (IFC) is an association of manufacturers and marketers of formulated nutrition products (e.g., infant formulas and adult nutritionals) whose members are based predominantly in North America. IFC members support the American Academy of Pediatrics' (AAP) position that breastfeeding is the preferred method of feeding infants. We also agree with the AAP that, for infants who do not receive breast milk, iron-fortified infant formula is the only safe and recommended alternative, IFC members are committed to providing infant formulas of the highest quality for those mothers who cannot or choose not to breastfeed, discontinue breastfeeding prior to one year or choose to supplement.

This petition seeks to add L-Methionine to the National List to permit its addition as a nonagricultural ingredient in infant formula based on isolated soy protein. L-Methionine is an essential amino acid for humans of all ages. Amino acids are the building blocks of protein. An essential amino acid is one that must be provided in the foods in our diet since our bodies do not have the capability of producing enough of it for normal metabolism and growth. Supplementing isolated soy protein with L-methionine improves the biological value of the protein and makes it nutritionally similar to breast milk protein and the protein of milk-based infant formula in its ability to sustain normal growth and development of young infants.

L-Methionine must be added to infant formulas based on isolated soy protein to satisfy the protein biological quality requirement in the U.S. Food and Drug Administration (FDA) regulation for infant formula [21 CFR 107.100(f)] and to support normal growth and development of full-term infants fed soy isolate infant formulas. Only the L-form of methionine is permitted in infant food by FDA regulation [21 CFR 172.230]. Thus, L-methionine is necessary for the production of the organic product identified as infant formula based on isolated soy protein.

Our petition and its appendices provide answers to all of the questions in the Guidelines on Procedures for Submitting National List Petitions, and in a manner that satisfies the criteria in the OFPA. We are available to provide any additional information that is required to complete your review process and recommendation.

Sincerely,

Mardi K. Mountford, MPH
Executive Vice President

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Petition for Inclusion of L-Methionine on the National List for Use in Infant Formula Based on Isolated Soy Protein

Item A

The petitioned substance L-Methionine will be included on § 205.605, Non-agricultural (non-organic) substances allowed in or on processed products labeled as ‘‘organic’’ or ‘‘made with organic (specified ingredients),’’ with the annotation “for use only in infant formula based on isolated soy protein.”

Item B

1. The substance’s chemical or material common name.

The name of the substance is (L)-Methionine. L-Methionine is an essential amino acid for humans of all ages. Amino acids are the building blocks of protein. An essential amino acid is one that must be provided in the foods in our diet since our bodies do not have the capability of producing enough of it for normal metabolism and growth.

Synonyms for L-methionine include the following:

(S)-2-Amino-4-(methylthio)butanoic acid

2-Amino-4-(methylthio)butyric acid, (S)-

2-Amino-4-methylthiobutanoic acid (S)-

Butanoic acid, 2-amino-4-(methylthio)-, (S)-

L(-)-Amino-gamma-methylthiobutyric acid

L-alpha-Amino-gamma-methylmercaptobutyric acid

L-alpha-Amino-gamma-methylthiobutyric acid

L-gamma-Methylthio-alpha-aminobutyric acid

It is important to stress that the form of methionine used in human infant nutrition must be the natural “L-form,” the physiologically occurring form of methionine. Use of the unnatural “D-form” in the present application is prohibited by the FAO/WHO Codex Alimentarius Commission Standard for infant formula and by FDA regulation (21 CFR 172.230: “DL-Methionine (not for infant foods)”).

2. The manufacturer’s or producer’s name, address and telephone number and other contact information of the manufacturer/producer of the substance listed in the petition.

Two manufacturers currently certified as suppliers of L-methionine are Sekisui Medical Company and Evonik-Rexim Pharmaceutical Company.

Sekisui Medical Company
KDX Nihombashi 313 building 5F
3-13-5, Nihombashi, Chuo-ku, Tokyo, 103-0027
Tel.: +81-3-3272-0671 / Fax: +81-3-3278-8774
http://www.sekisuimedical.jp/

Evonik Rexim (Nanning) Pharmaceutical Co., Ltd
No.10, Wenjiang Road
Wuming County
530100 Nanning, China
c/o Evonik Degussa Corp. USA
379 Interpace Parkway
Parsippany,NJ 07054
Tel.: 973-541-8000 / Fax: 973-541-8040

3. The current use of L-Methionine is as a nutritionally essential amino acid used to improve the biological value of infant formula based on isolated soy protein.

4. L-Methionine is currently used as an ingredient in infant formulas based on isolated soy protein.

Isolated soy protein contains sufficiently less of the essential amino acid methionine than do the proteins in breast milk and milk-based infant formula to make methionine the “limiting essential amino acid” (the essential amino acid in lowest relative amount for adequate growth and development) of isolated soy protein. Supplementing isolated soy protein with L-methionine improves the biological value of the protein and makes it nutritionally equivalent to breast milk protein and the protein of milk-based infant formula in its ability to sustain normal growth and development of young infants.

5. The source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.

The following information is excerpted and adapted from the description of L-methionine in the Hazardous Substances Data Base prepared by the National Library of Medicine and taken from an authoritative and reliable source[1].

The production method of choice for L-methionine is the enzymatic resolution of racemic N-acetyl-DL-methionine using acylase from Aspergillus oryzae. The production is carried out in a continuously operated fixed-bed or enzyme membrane reactor. Alternatively, L-methionine may be produced by microbial conversion (fermentation) of the corresponding 5-substituted hydantoin. Growing cells of Pseudomonas sp. strain NS671 convert DL-5-(2-methylthioethyl)hydantoin to L-methionine; a final concentration of 34 g/L and a molar yield of 93% have been obtained.

[1] Eggersdorfer M et al; Ullmann's Encyclopedia of Industrial Chemistry 7th ed. (2008). New York, NY: John Wiley & Sons; Vitamins.

The most economic way for production of DL-methionine is the chemical process based on acrolein, methyl mercaptan, hydrogen cyanide, and ammonium carbonate. β-Methylthiopropionaldehyde, formed by addition of methyl mercaptan to acrolein, is the intermediate that reacts with hydrogen cyanide to give alpha-hydroxy-gamma-methylthiobutyronitrile. Treatment with ammonium carbonate leads to 5-(β-methylthioethyl)hydantoin that is saponified by potassium carbonate giving DL-methionine in up to 95% yield, calculated on acrolein.

Additional synthetic production processes for DL-methionine are mentioned in the 2001 TAP Review for DL-methionine usage in organic livestock production. See page 3, Appendix A.

Supplier Evonik Rexim uses an enzymatic process for the final step in the production of L-methionine.

Supplier Sekisui Medical Co. synthesizes DL-methionine via hydantoin intermediates and then isolates the L-amino acid by enzymatic resolution.

6. A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance.

The National List currently includes DL-methionine, DL-methionine hydroxyl analog, and DL-methionine hydroxyl analog calcium, as feed additives for use in organic poultry production, at §205.603(d)(1). The National Organic Standards Board Technical Advisory Panel Review for the USDA National Organic Program on methionine for livestock use dated May 21, 2001 is attached as Appendix A.

NOP posted a Final Rule on synthetic Methionine in organic livestock production on March 14, 2011 that extended the allowance for methionine in organic poultry production until October 1, 2012, at the following maximum allowable limits of methionine per ton of feed: 4 pounds for layers, 5 pounds for broilers, and 6 pounds for turkeys and all other poultry.

Critically, the sources of methionine allowed on the National List for livestock (poultry) include the racemic DL-form and two synthetic analogs of methionine, all three of which are NOT allowed in infant formula products.

The L-form of methionine has not been petitioned or formally reviewed for use in organic handling of infant formula based on isolated soy protein.

The report in the National Library of Medicine Hazardous Substances Data Bank is attached as Appendix B

7. Regulatory information

The FDA regulates the use in foods of amino acids, including L-methionine, at 21 CFR 172.320; see Appendix C. L-Methionine is a food additive permitted for direct addition to food for human consumption, as long as 1) the quantity of the substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive, or other technical

effect in food, and 2) any substance intended for use in or on food is of appropriate food grade and is prepared and handled as a food ingredient.

The FDA promulgates the Infant Formula regulations under the authority of the Infant Formula Act. 21 CFR 107.100(f) requires a minimum biological quality for the protein in infant formula. As noted below in Items B.11 and B.12, the addition of L-methionine to infant formula based on isolated soy protein is required for normal growth and development of the young infant and to achieve the minimum biological quality required at 21 CFR 107.100(f), which is provided in Appendix C.

The FAO/WHO Codex Alimentarius Commission created a Codex Standard for infant formula over thirty years ago. Two elements of this standard are relevant to this petition. The standard requires a minimum biological quality of the protein in infant formula; isolated soy protein requires L-methionine addition to achieve the minimum. The Codex standard also requires use of the natural L-form of an amino acid, including methionine. The latest Codex standard for infant formula also is included in Appendix C.

8(a). The Chemical Abstract Service (CAS) number of L-methionine is 63-68-3.

8(b). The label of the currently marketed organic infant formula product that contains L-methionine is attached as Appendix D.

9. The substance's physical properties and chemical mode of action.

Physical Properties:

Physical state and appearance:	Powdered solid
Color:	White
Odor:	Slight
Taste:	Sulfurous
Molecular Weight:	149.21 g/mole
Solubility:	Soluble in water, warm dilute alcohol
pH (1% solution in water):	5.85 (slightly acidic)
Melting Point:	281°C (537.8°F)

Mode of Action: L-methionine is an indispensable amino acid. Humans, as well as other non-ruminant mammals, cannot fix inorganic sulfur into organic molecules and must rely on ingested sulfur amino acids, such as methionine, for the synthesis of protein and biologically active sulfur.

L-Methionine is currently used as a nutritionally essential amino acid required to improve the biological value of a marketed organic infant formula based on isolated soy protein. L-Methionine has been added to conventional soy isolate based infant formulas in the U.S. for over forty-five years.

(a) Chemical interactions with other substances, especially substances used in organic production.

DL-methionine is allowed on the National List for use in poultry rations, to improve the nutritional quality of soybean-based rations. L-methionine also can be used for this purpose. L-methionine is an unreactive powder that easily blends into dry mixes and is soluble in water, especially warm or hot water, so it can be disperse in wet mashes.

(b) Toxicity and environmental persistence (Source of data: Hazardous Substances Data Bank; see Appendix B).

Human Toxicity: Based on distribution data from the 1984-1994 NHANES III, the mean daily intake for all life stage and gender groups of methionine from food and supplements is 1.8 grams per day. Men 51 though 70 years of age had the highest intakes at the 99th percentile of 4.1 grams per day.[2]

Methionine supplements (5 g/day) for periods of weeks were reportedly innocuous in humans. Single oral doses of 7 g produced lethargy in six individuals and oral administration of 10.5 g of L-methionine to one produced nausea and vomiting.

Non-Human Toxicity: L-Methionine is an essential amino acid for rats, mice, chickens, and swine, as well as for humans. L-methionine needs to be furnished along with other essential amino acids for it to be incorporated into the proteins needed for normal growth and development. A diet devoid of methionine does not sustain life. Conversely, administering a large, non-physiological level of L-methionine, in the absence of other essential amino acids, can create metabolic imbalance and toxicity.

A single dietary dose (2.7% of the diet) of L-methionine decreased body growth and also reduced food intake in rats. Dietary excesses of L-methionine (2.7% of the diet) for 6, 13, or 20 days have been associated with erythrocyte engorgement and accumulation of hemosiderine in rats, and there was a depression of growth and splenic damage. Dietary intakes of 2 to 4% of L-methionine caused slight changes in liver cells in rats and slight decreases in liver iron content.

[2] Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Institute of Medicine, National Academies Press, 2005, page 725.

Darkened spleens caused by increases in iron deposition have been observed in weanling rats fed 1.8% methionine diets for 28 days. Male Wistar rats were fed either an L-methionine-supplemented (2.5 g/100 g) diet without changing any other dietary components or a control (0.86 g/100 g) diet for 7 weeks. L-Methionine supplementation in the diet specifically increases mitochondrial ROS production and mitochondrial DNA oxidative damage in rat liver mitochondria offering a plausible mechanism for its hepatotoxicity.

Environmental Persistence: L-Methionine is formed in natural waters through metabolism of naturally occurring proteins. It is one of the nine indispensable amino acids that cannot be synthesized to meet body needs in animals and therefore must be provided in the diet. L-methionine is not expected to adsorb to suspended solids and sediment. The potential for bioconcentration in aquatic organisms is low. Using a laboratory activated sludge system, L-methionine exhibited an 80% theoretical BOD reduction in 16 days.

L-Methionine has been shown to degrade in sunlit natural water through a photo-sensitized oxidation involving singlet oxygen. Assuming that the top meter of sunlit natural water has a singlet oxygen concentration of 4X10-14 M, the photooxidation half-life for the reaction L-methionine with singlet oxygen has been estimated to be about 200 hr at pH 6-11. The near-surface photooxidation rate (via singlet oxygen) of L-methionine in Okefenokee Swamp water from Georgia is predicted to be about 3 hr. Bioconcentration and volatilization are not expected to be important fate processes because of its high water solubility.

(c) Environmental impacts from its use and/or manufacture.

L-methionine is an indispensable amino acid that cannot be synthesized in the body of animals and therefore must be provided in the diet. L-Methionine is used in normal metabolism and is incorporated into the protein of every living organism on the earth. This accounts for its rapid biological degradation in aquatic systems (See Item 9.b.)

In 2005, the environmental impact of the use and manufacture of synthetic methionine was described in correspondence from Degussa (predecessor of Evonik) to NOP that is available on the NOP website[3] and is included in Appendix B.

The manufacturing plant of Evonik Rexim in Wuming, China, is ISO-certified and FDA-inspected and operates according to HAACP (Hazard Analysis/Critical Control Points) requirements. Sustainable development is an integral part of the business process. Economic, ecologic, and societal aspects are given equal consideration.

The manufacturing plant of Sekisui Medical Co. for L-methionine is Iwate Factory in Hachimantai City, approximately 600 km north of Tokyo. Sekisui actively takes steps to preserve the environment. They reached a pollution prevention agreement with Hachimantai city (former Matsuo village) in 1977, and constructed an anaerobic fermentation processing facility in 1992. They obtained ISO14001 certification in Spring 2001.

[3] http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRD3479561 accessed 7 August 2011.

(d) Effects on human health.

L-Methionine is an essential, indispensable amino acid. Humans cannot fix inorganic sulfur into organic molecules and must rely on ingested sulfur amino acids, such as methionine, for the synthesis of protein and biologically active sulfur compounds.

L-Methionine has other, non-nutritional uses. It is used as a hepatoprotectant (liver protector) and as an antidote to acetaminophen poisoning, the result of which is liver damage.

(e) Effects on soil organisms, crops, or livestock.

Poultry have a greater need for this essential sulfur-containing amino acid than do other food and fiber livestock sources, because they have feathers. DL-methionine is a customary ingredient in poultry rations. L-Methionine can replace the DL-form in this application.

10. Safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies.

An MSDS is attached in Appendix E. The Hazardous Substances Data Bank information on L-methionine prepared by the National Library of Medicine is included in Appendix B.

11. Research information about L-Methionine, including comprehensive substance research reviews and research bibliographies.

General nutritional research information for L-methionine has been summarized by the Institute of Medicine in 2005[4], and is included in Appendix F.

This petition requests the allowance on the National List of L-methionine solely for use in infant formula based on isolated soy protein. Consequently, this section of the petition will focus on research information and comprehensive research reviews about this use.

Infant formulas based on isolated soy protein were first introduced around 1965, following the commercial introduction of high quality isolated soy protein raw materials. Edible soy protein is isolated from defatted soy flour or soy ‘flakes’ by dispersing the flour or flakes in mildly alkaline water containing sodium hydroxide or potassium hydroxide at a pH less than 9 (a baking soda (sodium bicarbonate) solution has a pH of about 8.4). The extract is clarified to remove the insoluble material – insoluble protein, fiber – and the "supernatant" is then acidified to a pH range of 4-5. The globular proteins precipitate as curd. The protein-curd is collected and separated from the soy whey by centrifugation. The curd may be neutralized (solubilized) with sodium hydroxide or potassium hydroxide prior to drying to yield a soluble proteinate powder.

[4] Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Institute of Medicine, National Academies Press, 2005.

Conventional soy protein isolate[5] is produced from hexane-extracted soy flakes and the acidifier is hydrochloric acid; both hexane and hydrochloric acid are unacceptable in an organic process. One commercial process[6] for organic soy protein isolate uses carbon dioxide to "de-fat" full-fat soy flour and citric acid for pH adjustment. The organic isolated soy protein[7] used in the currently marketed organic infant formula shown in Appendix D is produced from mechanically pressed soybeans, with citric acid being used as the acidifier. All these isolated soy proteins have substantially identical amino acid profiles, confirming that they contain the same globular protein fraction of the soybean.

The fact that each of these isolated soy proteins represent the same protein fraction of the soybean means that the infant nutritional research on conventional soy protein isolates published over the past 50 years is applicable to the more recently developed organic isolated soy protein and organic soy protein isolate.

Federal regulations[8] require that the protein efficiency ratio (PER) of the nitrogen source of an infant formula be at least 70% of that of casein, a standard protein. Isolated soy proteins used in infant formulas are supplemented with L-methionine, the limiting amino acid. The extent of supplementation is that necessary to meet the requirements of FDA with respect to the PER.[9]

Clinical evidence supports the addition of L-methionine to infant formula based on isolated soy protein.

Fomon et al. (1979)[10] compared the data of nine normal full-term infants fed a soy isolate-based formula unsupplemented with methionine with similar data from 10 similar infants fed the same formula supplemented with L-methionine. Statistically significant differences were as follows: lesser weight gain per 100 kcal by infants fed the unsupplemented soy isolate-based formula than by infants fed milk-based or other soy isolate-based formulas; lesser serum concentrations of albumin at age 28 days by infants fed the unsupplemented soy isolate-based formula than by breast-fed infants; greater serum concentrations of urea nitrogen by infants receiving the unsupplemented soy isolate-based formula than by those receiving the same formula supplemented with L-methionine. The results suggest that normal infants fed a formula providing 2.25 /100 kcal of isolated soy protein not fortified with methionine perform less well during the first 6 weeks of life than do breast-fed infants and infants fed milk-based formulas or

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[5] The commercial definition of "protein isolate" is a material with no less than 90% protein, dry matter basis.The isolated protein extracted from hexane-defatted soy flakes contains very little fat.
[6] U.S. Patent Application 20070207255, published September 6, 2007: "Plant-derived protein compositions."
[7] Mechanically pressing is not as efficient a means for removing soy oil from soybeans as is hexane extraction. Consequently, the soy protein material resulting from this organic process contains about 15% oil and thus less than 90% protein, and it cannot be designated "soy protein isolate."
[8] 21 CFR 107.100(f).
[9] Nutrition of Normal Infants. S. J. Fomon. 1993. Mosby, St. Louis. Page 126.
[10] Fomon SJ, Ziegler EE, Filer LJ, Jr., Nelson SE & Edwards BB (1979) Methionine fortification of a soy protein formula fed to infants. *Am J Clin Nutr* **32**, 2460-2471.

formulas based on isolated soy protein fortified with methionine. The limiting nutrient appears to have been methionine. A copy of this study can be found in Appendix G.

In another study by Fomon et al. (1986)[11], measurement of weight gain and serum concentrations of urea nitrogen and albumin clearly showed a beneficial effect of methionine supplementation at protein concentrations of 2.2 and 2.6 g/100 kcal. This is relevant to the currently marketed organic infant formula based on isolated soy protein (label shown in Appendix D), which provides protein at 2.5 g/100 kcal. A copy of this study also can be found in Appendix G.

A major authoritative body, the Committee on Nutrition of the European Society for Pediatric Gastroenterology and Nutrition, reviewed soy isolate infant formulas twice in the past twenty-five years. These two reviews also can be found in Appendix G. In 1990, their statement[12] read:

> Isolated soy protein if appropriately processed is a good vegetable protein source for children (7). It has a high nutritional value and its amino acid composition rating is 96% that of casein, and even after allowance has been made for digestibility, the amino acid score is 89% overall and still remains above 80% when the least available amino acid, methionine, is considered, but nevertheless this is limiting (8). Thus even when protein intake is not marginal, methionine supplements are needed to ensure growth, and to maintain nitrogen balance and circulating plasma albumin concentrations (9). The Committee considers, therefore, that soy protein isolate based infant and follow-up formulas should contain at least 30 mg (200 pmol) of methionine/100 kcal (50 pmol (7.3 mg)/100 kJ), approximating to the amount in human breast milk.

In 2006, the ESPGHAN Committee on Nutrition[13] wrote:

> Soy protein isolates are derived from delipidated soy flour (90-95%) by elimination of soluble carbohydrates and mineral salts (5). Soy protein has a lower biologic value than cows' milk protein. The nitrogen conversion factor, which allows us to calculate the protein content from the total nitrogen content, is lower for soy protein isolate than for cows' milk protein. Soy and cows' milk proteins have a different amino acid pattern (i.e., soy protein contains lower amounts of methionine, branched chain amino acids lysine, and proline and higher quantities of aspartate, glycine, arginine, and cystine than cows' milk protein) (14). To ensure adequate growth, nitrogen balance, and plasma albumin concentrations, methionine supplements have been recommended (15,16).

The Committee specifically set a minimum of 2.25 grams of protein per 100 kcal and a minimum L-methionine level of 29 mg/100 kcal for infant formula based on isolated soy protein.

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[11] Fomon SJ, Ziegler EE, Nelson SE & Edwards BB (1986) Requirement for sulfur-containing amino acids in infancy. *J Nutr* **116**, 1405-1422.

[12] Aggett, P. J., F. Haschke, et al. (1990). "Comment on the composition of soy protein based infant and follow-up formulas. ESPGAN Committee on Nutrition." Acta Paediatr Scand **79**(10): 1001-1005.

[13] Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KF, Puntis J, Rieu D, Rigo J, Shamir R, Szajewska H & Turck D (2006) Soy protein infant formulae and follow-on formulae: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* **42**, 352-361.

12. Petition Justification Statement for Inclusion of the Synthetic L-Methionine on the National List at §205.605(b)

L-Methionine must be added to infant formulas based on isolated soy protein to satisfy the protein biological quality requirement in the FDA regulation for infant formula [21 CFR 107.100(f)] and to support normal growth and development of full-term infants fed soy isolate infant formulas. Only the L-form of methionine is permitted in infant food by FDA regulation [21 CFR 172.230]. Thus, L-methionine is necessary for the production of the organic product identified as infant formula based on isolated soy protein.

Currently, all commercially available L-methionine is made from synthetic intermediates, followed either by a final fermentation or by enzymatic resolution, According to our interpretation of the definition of synthetic at §205.2, this L-methionine is synthetic.

L-Methionine is found naturally in proteins. Many L-amino acids are produced commercially by fermentation, a process that **may** result in the L-amino acid being nonsynthetic. In 2011, it was announced[14] that a new industrial bio-fermentation process to produce L-methionine from plant-based raw materials would be industrialized in Southeast Asia, at the end of 2013. However, the announcement also included a reference to the production of methyl mercaptan, a sulfur-based intermediate said to be key to the manufacture of methionine. Using this synthetic material in the bio-fermentation process would make this L-methionine synthetic, as we read the definition in §205.2.

The justification for soy-based infant formula is clearly described in “Infant Nutrition and Feeding – A reference Handbook for Nutrition and Health Counselors in the WIC and CSF Programs,” a resource published by the Food and Nutrition Service of USDA [Publ. No. FNS-288][15].

> Soy-Based Infant Formula
>
> Soy-based infant formulas were developed for infants who cannot tolerate infant formula made from cow’s milk. These infant formulas contain soy protein isolate made from soybean solids as the protein source, vegetable oils as the fat source, added carbohydrate (usually sucrose and/or corn syrup solids), and vitamins and minerals. Soy-based infant formulas are fortified with the essential amino acid methionine, which is found in very low quantities in soybeans. In these infant formulas, 10 to 11 percent of the kilocalories are provided by protein, 45 to 49 percent by fat, and 41 to 43 percent by carbohydrate. All soy-based infant formulas are fortified with similar amounts of iron as milk-based iron-fortified infant formulas.
>
> The AAP[16] has stated that soy-based infant formulas are safe and effective alternatives to cow’s milk-based infant formulas, but have no advantage over them.

[14] http://www.arkema.com/pdf/EN/press_release/2011/cj_and_arkema_press_release_va.pdf
[15] http://www.nal.usda.gov/wicworks/Topics/FG/Chapter4_InfantFormulaFeeding.pdf. Accessed 28 September 2011.
[16] The American Academy of Pediatrics

Soy-based infant formulas may be indicated in the following situations:

- Infants with galactosemia (a rare metabolic disorder) or hereditary lactase deficiency
- Infants whose parents are seeking a vegetarian diet for their full-term infant or
- Infants with documented IgE-mediated allergy to cow's milk protein.

The use of soy-based infant formulas has no proven benefit in the following situations:

- Healthy infants with acute gastroenteritis in whom lactose intolerance has not been documented
- Infants with colic
- Prevention of allergy in healthy or high-risk infants, and
- Infants with documented cow's milk protein-induced enteropathy or enterocolitis.

A larger question relates to justifying the need for infant formula. Breast milk is universally recognized as the preferred feeding for infants in the first year of life (at least). Breast feeding should be the exclusive nutrition of the infant for the first four to six months of life and the nutritional foundation for the rest of the first year of life, when the infant begins to become accustomed to the tastes and textures of the foods consumed by the rest of the family.

Given the nutritional superiority of breast milk and its general benefits, which range from higher IQ to better speech enunciation, why is infant formula needed? The answer is found in the Breastfeeding Report Card of the United States, 2010[17], the latest year available, which shows that only 3 out of every 4 new mothers in the U.S. starts out breastfeeding. Rates of breastfeeding at 6 and 12 months as well as the rate of exclusive breastfeeding at 3 months are discouragingly low, as the following chart shows.

	Ever Breastfed	Breastfeeding at 6 months	Breastfeeding at 12 months	Exclusive breast-feeding at 3 months
U.S. National	75.0%	43.0%	22.4%	33.0%

These data demonstrate the need for infant formula as a substitute for human milk in meeting the normal nutritional requirements of infants who are not receiving only breast milk. Infant formulas must conform to the regulations at Parts 106 and 107, Title 21, Code of Federal Regulations, which require that an infant formula shall be nutritionally adequate to promote normal growth and development when use in accordance with its directions for use.

13. A Confidential Business Information Statement

This petition contains no Confidential Business Information.

[17] http://www.cdc.gov/breastfeeding/pdf/BreastfeedingReportCard2010.pdf Accessed September 27, 2011.

Appendices

Petition for addition of the Synthetic Substance L-METHIONINE to 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 National Organic Standards Board Technical Advisory Panel Review of DL-Methionine in Organic Livestock Production for the USDA National Organic Program, May 21, 2001

Appendix B Toxicology and Environmental Information

- Hazardous Substances Data Bank – L-Methionine
- 2005 Letter and Enclosure from Degussa Corporation to Arthur Neal, NOP

Appendix C U.S. Regulations and International Standards

- L-Methionine regulation - CFR 172.320
- Infant Formula regulations - 21 CFR 107.100(f) biological quality
- Codex Alimentarius Commission Standard 72-1981

Appendix D Product Label

Appendix E L-Methionine Material Safety Data Sheet (MSDS)

Appendix F Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Institute of Medicine, National Academies Press, 2005. Pp. 725-6.

Appendix G Pediatric Research Studies and Reviews

- Fomon SJ, Ziegler EE, Filer LJ, Jr., Nelson SE & Edwards BB (1979) Methionine fortification of a soy protein formula fed to infants. *Am J Clin Nutr* **32**, 2460-2471.
- Fomon SJ, Ziegler EE, Nelson SE & Edwards BB (1986) Requirement for sulfur-containing amino acids in infancy. *J Nutr* **116**, 1405-1422.
- Aggett PJ, Haschke F, Heine W, Hernell O, Launiala K, Rey J, Rubino A, Schoch G, Senterre J & Tormo R (1990) Comment on the composition of soy protein based infant and follow-up formulas. ESPGAN Committee on Nutrition. *Acta Paediatr Scand* **79**, 1001-1005.
- Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KF, Puntis J, Rieu D, Rigo J, Shamir R, Szajewska H & Turck D (2006) Soy protein infant formulae and follow-on formulae: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* **42**, 352-361.

for the USDA National Organic Program

May 21, 2001

Methionine
Livestock

Executive Summary

The NOSB received a petition in 1995 to add all synthetic amino acids to the National List. After deliberation of a review prepared by the TAP in 1996 and 1999, the NOSB requested a case-by-case review of synthetic amino acids used in livestock production, and referred three forms of methionine to the TAP.

All of the TAP reviewers found these three forms to be synthetic. Two TAP reviewers advised that synthetic methionine remain prohibited. The one reviewer who advises the NOSB to recommend adding synthetic methionine to the National List agrees that it is not compatible with organic principles and suggests limitations on its use until non-synthetic sources are more widely available.

The majority of the reviewers advise the NOSB to not add them to the National List for the following reasons:

1) Adequate organic and natural sources of protein are available [§6517(c)(1)(A)(ii)];
2) Methionine supplementation is primarily to increase growth and production, not to maintain bird health, and this is counter to principles embodied in the OFPA requirements for organic feed [§6509(c)(1)];
3) Pure amino acids in general and synthetic forms of methionine in particular are not compatible with a sustainable, whole-systems approach to animal nutrition and nutrient cycling [§6518(m)(7)].

Methionine is an essential amino acid needed for healthy and productive poultry. It is generally the first limiting amino acid in poultry diets. Synthetic ("pure") amino acids are produced either synthetically or from genetically engineered sources and involve the use of highly toxic and hazardous chemicals such as hydrogen cyanide, ammonia, and mercaptaldehyde. Synthesis of DL-methionine, and DL-methionine hydroxy analogs also result in significant pollution of the environment. These sources of methionine do not occur in nature.

Most amino acids are metabolized from protein, even in conventional feeding situations. Adequate levels of essential amino acids can be obtained in the diet of poultry fed adequate levels of intact protein from natural sources. Synthetic amino acids are used to improve feed conversion efficiency and lower feed costs.

Although there may be limitations in the current supply of diverse organic protein sources, a requirement for natural, non-GMO sources of methionine will stimulate market development in organic and approved feedstuffs. Other natural sources, such as fish meal, crab meal, and yeast are also available, and would be more compatible with organic standards than synthetic ones. Clarification of the status of some of these alternatives is needed. If synthetic substances are allowed to substitute for organic feed, that undermines the incentive to produce organic feedstuffs.

Humans have raised poultry for centuries without synthetic amino acids. Synthetic amino acids have become part of the standard poultry diet only over the past 50 years or so as production has moved from extensive pasture-based nutrition to high-density confinement systems.

Reliance on a higher protein diet to achieve necessary amino acid balance may result in higher excretion of uric acid that can form ammonia in the litter. Under an organic management system where there is access to the outdoors, suitable densities, and integrated management of manure and crop production this is not a problem. "Excess" nitrogen is not a waste problem in an organic system; it is a valuable resource that needs to be managed in an integrated and holistic way.

Livestock

Identification

Chemical Names:
2-amino-4-methylthiobutyric acid and
α-amino-γ-methylmercaptobutyric acid

Other Names:
DL-methionine, D-methionine, L-methionine, Met, Acimethin

CAS Numbers:
59-51-8 (DL-methionine)
63-68-3 (L-methionine)
348-67-4 (D-methionine)

Other Codes:
International Feed Names (IFN):
DL-methionine: 5-03-86
DL-methionine hydroxy analog calcium: 5-03-87
DL-methionine hydroxy analog: 5-30-28

Poultry Production

Background:
This supplementary information was requested by the NOSB to be added to the 1999 Technical Advisory Panel general review of amino acids for use in livestock production. The NOSB tabled the decision in October of 1999 on amino acids for all livestock, and decided to consider only the use of methionine for poultry production. Supporting information for this use was received (Krengel, 2001) and this additional information is addressed in this review in addition to a more recent review of the literature.

Summary of TAP Reviewer Analysis [1]

Synthetic / Non-Synthetic:	Allowed or Prohibited	Suggested Annotation:
Synthetic (3-0)	Prohibit (2)	None.
	Allow (1)	Source must be non-GM. For poultry rations only. Only when alternatives are not available including ration diversity and acceptable animal and plant sources, or enzyme digested natural protein sources. Not to exceed 0.1% by weight in any feed source directly fed to poultry.

Characterization

Note: The description of composition, properties, and manufacturing process (how made) remains the same as provided in the 1999 TAP review. The sections on specific uses, action, combinations, regulatory status, and review under OFPA criteria 1,2,5,6, and 7 have been revised to consider poultry production.

Composition:
Amino acids have an amino group (NH_2) adjacent to a carboxyl (COOH) group on a carbon. The model amino acid for livestock production is methionine. The formula for methionine is $H_2NCH_3SCH_2CH_2COOH$.

Properties:

[1] *This Technical Advisory Panel (TAP) review is based on the information available as of the date of this review. This review addresses the requirements of the Organic Foods Production Act to the best of the investigator's ability, and has been reviewed by experts on the TAP. The substance is evaluated against the criteria found in section 2119(m) of the OFPA [7 USC 6517(m)]. The information and advice presented to the NOSB is based on the technical evaluation against that criteria, and does not incorporate commercial availability, socio-economic impact or other factors that the NOSB and the USDA may want to consider in making decisions.*

L-Methionine: Colorless or white lustrous plates, or a white crystalline powder. Has a slight, characteristic odor. Soluble in water, alkali solutions, and mineral acids. Slightly soluble in alcohol, insoluble in ether. MP 280-282°C. It is assymetric, forming both an L- and a D- enantiomer. Methionine hydroxy analog (MHA) is available in liquid form.

How Made:

Methionine may be isolated from naturally occurring sources, produced from genetically engineered organisms, or entirely synthesized by a wide number of processes. While methionine has been produced by fermentation in laboratory conditions, racemic mixtures of D- and L- methionine (DL-Methionine) are usually produced entirely by chemical methods (Araki and Ozeki, 1991). Methionine can be produced from the reaction of acrolein with methyl mercaptan in the presence of a catalyst (Fong, et al., 1981). Another method uses propylene, hydrogen sulfide, methane, and ammonia to make the intermediates acrolein, methylthiol, and hydrocyanic acid (DeGussa). The Strecker synthesis can be used with α-methylthiopropionaldehyde as the aldehyde (Fong, et al., 1981). A recently patented process reacts 3-methylmercaptopropionaldehyde, ammonia, hydrogen cyanide, and carbon dioxide in the presence of water in three reaction steps (Geiger et al., 1998). Other methods are discussed in the 1999 Crops Amino Acid TAP review. DL-methionine hydroxy analog calcium and DL-methionine hydroxy analog forms are considered to be alpha-keto acid analogues in which the amine group has been replaced by a hydroxy (OH) group. These forms are converted to the amino form in the bird by transamination in the liver, using non-essential amino acids such as glutamic acid (Cheeke 1999; Leeson 1991). These forms are produced by reacting hydrogen cyanide with an aldehyde that has been treated with a sulfite source to form a cyanohydrin. The aldehydes used are prepared from either hydrogen sulfide or an alkyl mercaptan with an aldeyhde such as acrolein and are then hydrolyzed using sulfuric or hydrochloric acid (USPO 1956).

Specific Uses:

The requested use for methionine in poultry production is as a feed supplement. For optimum health and performance, the animal's diet must contain adequate quantities of all nutrients needed, including amino acids. The essential amino acid furthest below the level needed to build protein is known as the limiting amino acid. A shortage of the limiting amino acid will constrain animal growth, reduce feed efficiency, and in extreme cases cause a nutritional deficiency. Supplementation with isolated amino acids increases feed conversion efficiency, thus lowering feed costs per unit of weight gain or production (Pond, Church, and Pond, 1995). Methionine is considered to be the first limiting amino acid in corn-soy poultry diets, followed by lysine and arginine (Baker 1989, NRC 1994, Cheeke 1999). An extensive literature has been published that documents the efforts to optimize the balance of amino acids in poultry diets in order to lower costs, reduce need for animal or fish proteins, replace soy meal with less expensive or more locally available plant proteins, and utilize plant proteins more efficiently (De Gussa 1995, 1996; North 1990; Neto, et. al. 2000; Cino 1999; Emmert 2000; DiMello 1994; Weibel 2000).

Amino acids are also used in livestock health care. Methionine is used as a urine acidifier because excretion of its sulfate anion lowers urine pH. Its sulfate anion may also displace phosphate from magnesium-ammonium-phosphate hexahydrate (struvite, double phosphate, or triple phosphate if calcium is also present) crystals and uroliths, which form best at a pH above 6.4-6.6. As a result of these effects, methionine is used to assist in dissolving and/or preventing uroliths, kidney stones, bladder stones, or urologic syndromes thought to be caused by struvite uroliths or crystals (Lewis, Morris, and Hand, 1987). Methionine is also used to assist in the treatment and/or prevention of hepatic lipidosis because of its need for body fat mobilization and transport.

Action:

Amino acids form protein. Of the 22 amino acids found in body proteins, the National Research Council lists 13 as essential in poultry diets, and these must be consumed in feed. These 13 are: arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, proline, threonine, tryptophan, and valine (NRC 1994). Five that are deemed critcial in poultry rations are methionine, cystine, lysine, tryptophan, and arginine (North, 1990).

Animals convert dietary protein into tissue protein through digestive processes. Proteins are metabolized by animals through two phases: catabolism (degradation from body tissue to the free amino acid pool) and anabolism (synthesis into body tissue). Amino acids utilized as proteins are primary constituents of structural and protective tissues, including skin, feathers, bone, ligaments, as well as muscles and organs.

Combinations:

Amino acids are combined in feed rations of grains, beans, oilseeds, and other meals with antioxidants, vitamins, minerals, antibiotics, and hormones (Pond, Church, and Pond, 1995). Methionine is a precursor in the diet to cystine, and the amount needed in the diet depends on the amount of cystine also present. Requirements for methionine are frequently cited in terms of methionine plus cystine, because methionine converts to cystine as needed.

Status

OFPA, USDA Final Rule

Amino acids do not appear on the list of synthetics that may be allowed according to the OFPA [7 USC 6517(c)(1)(B)(i)]. This list of permitted synthetics includes vitamins and minerals, livestock paraciticides, and medicines. Medicines can not be administered in the absence of illness (7USC 6509(d)(1)(C), and growth promoters including hormone, antibiotics, and synthetic trace elements may not be used to stimulate growth or production of livestock [7USC 6509(c)(3)]. Feed must be produced organically, and cannot contain synthetic nitrogen in the form of urea [7 USC 6509(c)]. Under the requirements of the USDA rule at 7CFR 205.237, synthetic substances added to feed must be listed under 205.603 or else will be prohibited at date of implementation.

Regulatory

Regulated as a nutrient / dietary supplement by FDA (21 CFR 582.5475). The Association of American Feed Control Officials (AAFCO) set the standard of identity for DL-methionine as containing a minimum of 99% racemic 2-amino-4-methylthiobutyric acid (AAFCO, 2001). The AAFCO model regulation states that "the term Methionine Supplement may be used in the ingredient list on a feed tag to indicate the addition of DL-Methionine" (AAFCO, 2001). AAFCO also lists a feed definition for DL-Methionine hydroxy analogue calcium (min. 97% racemic 2-amino-4-methylthiobutyric acid, 21 CFR 582.5477) and DL-Methionine hdyroxy analogue, (min. 88% racemic 2-amino-4-methylthiobutyric acid, 21 CFR 582.5477).

Status among Certifiers

Current published standards shows that two U.S. certifiers clearly prohibit amino acids (OCIA 2000; CCOF Intl. 2001) and some explicitly allow (FVO 2001; Oregon Tilth 1998; QAI 1999 - for nonruminants). Various other state and private certifiers either explicitly or implicitly allow the use of essential amino acids. The status among U.S. certifiers remains unresolved awaiting a recommendation by the NOSB.

Historic Use

The history of use in organic production is not clear due to lack of specific mention in most agency standards. The widepread use of crystalline (pure) amino acids in formulated rations has expanded greatly since 1980 for non-organic poultry production. Most current use in organic poultry production appears to be as a supplement for broilers (meat chickens) and turkeys as well as for laying hen feed rations.

International

CODEX - The Codex Draft Guidelines for Livestock production, approved May 2000 (Alinorm 01/22 Appendix II Annex 1. B. item 18) state that:

- feedstuffs of mineral origin, trace elements, vitamins, or provitamins can only be used if they are of natural origin. In case of shortage of these substances, or in exceptional circumstances, chemically well-defined analogic substances may be used;
- synthetic nitrogen or non-protein nitrogen compounds shall not be used.

This draft is considered to be at step 8 of the Codex process, and will go the Codex Alimentarius Commission in July 2001 for adoption (Joint FAO/WHO Standards Programme). This was considered to prohibit urea, (Lovisolo, 2001) and appears to also prohibit synthetic amino acids.[1]

EU 2092/91 - The European Standards do not include amino acids among permitted feedstuffs (European Union, 1999).

[1] Non-protein nitrogen compounds include substances such as urea and ammoniated materials (AAFCO, 2001). In the technical literature, non-protein nitrogen is considered to include "free amino acids, amino acid amides, glucosides containing nitrogen, nuleotides, urea, nitrates, ammonium salts and other low-molecular weight compounds containing nitrogen" (Boda, 1990).

IFOAM - Amino acids are prohibited for use in feed by IFOAM (IFOAM, 2000). IFOAM also supported the interpretation of the CODEX prohibition of non-protein nitrogen to extend to pure amino acids (Schmid, 2001).

Canada - Canadian standards allow essential amino acids, but explicitly prohibit ones from genetically engineered sources and state that the material may have some additional requirements. Operators are instructed to consult with their certification body for approval (Canadian General Standards Board, 1999).

OFPA 2119(m) Criteria

(1) The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.

The primary chemical interaction is the dietary intake by animals. While many of the interactions may be regarded as beneficial, excess methionine in a diet may cause deficiencies in other amino acids and induce toxicity (D'Mello, 1994). Methionine, while often one of the most limiting amino acids, is also one that readily goes to toxic excess. Small excesses of methionine can be deleterious (Buttery and D'Mello, 1994). Errrors in feed formulation or excess supplemental methionine can actually depress growth and development at levels of 40 g/kg (4.0%) (Baker, 1989, NRC 1994). Excess methionine exacerbates deficiencies of vitamin B-6, which results in depressed growth and feed intake (Scherer, 2000). Growth depressions resulting from excess supplemental amino acids include lesions in tissues and organs (D'Mello, 1994). Methionine is "well established as being among the most toxic of all amino acids when fed at excess levels in a diet" (Edmonds and Baker, 1987 cited in Scherer, 2000).

(2) The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.

While it is nutritionally essential, methionine excesses are far more toxic to poultry than similar excesses of tryptophan, lysine, and threonine (National Research Council, 1994). Force feeding methionine to excess can result in death to chicks (National Research Council, 1994). However, NRC acknowledges that such toxicities are unlikely in practical circumstances for poultry, in that an amino acid toxicity requires a particularly high level of an amino acid relative to all others. Supplemental levels fed to poultry are usually fed at lower levels, ranging from 0.3 - 0.5% of the diet. Susceptiblity of an animal to imbalances and excesses is influenced by the overall protein supply, and animals that are fed relatively high levels of protein are more tolerant (Buttery and D'Mello, 1994).

A dosage of 2 g / mature cat / day (20 to 30 g / kg dry diet) for 20 days induces anorexia, ataxia, cyanosis, methemoglobinemia and Heinz body formation resulting in hemolytic anemia (Maede, 1985). Rat studies of methionine is significantly toxic in excess (Regina, et al., 1993). High levels of methionine were found to be toxic to hepatic cells and liver function of the rat models. The results of this study indicated that the biochemical reason for the extreme sensitivity of mammals to excess dietary methionine is thought to be due to the accumulation of toxic catabolites, most notably, S-adenosylmethione, resulting in liver dysfunction. L-methionine has an acute LD_{50} of 4,328 mg/kg (rat) (NIEHS, 1999b). NIEHS carcinogenicity and teratogenicity are not available, but reports positive mutagenicity (NIEHS, 1999b).

Methionine is stable in crystalline form at standard temperature and pressure.

(3) *The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.*

Synthetic production of DL-methionine involves a number of toxic source chemicals and intermediates. Each of the several manufacturing processes used to produce DL-methionine was rated as either "moderately heavy" to "extreme" (Fong, et al., 1981). Newer processes have not replaced many of the feedstocks. Several of the feedstocks are likely to result in ruptured storage tanks, leaking chemicals, and releases into the environment. The methionine production process is listed by EPA as a hazardous air pollutant (40 CFR 63.184).

Methyl mercaptan can react with water, steam, or acids to produce flammable and toxic vapors (Sax, 1984). The EPA rates methyl mercaptan fires as highly hazardous and can cause death by respiratory paralysis (EPA, 1987). Acrolein has a toxicity rating of 5 (on a scale of 1 to 6 with 6 being most toxic) (Gosselin, 1984) and it is also an aquatic herbicide (Meister, 1999). The acrolein process involves several steps that render it synthetic as well (1994). Acrolein itself is an extreme irritant.

Hydrogen cyanide is produced by further processing of methane and ammonia. Hydrogen cyanide is a gas that is highly toxic. Hydrogen cyanide has a toxicity rating of 6 and is one of the fastest acting poisons known to man (Gosselin, 1984). Exposure causes paralysis, unconsciousness, convulsions, and respiratory arrest. Death usually results from exposure at 300 ppm concentrations for a few minutes. Manufacture of hydrogen cyanide is a significant source of atmospheric release of cyanide (Midwest Research Institute, 1993). Ammonia is a corrosive agent. Methane is a central nervous system depressant (Gosselin, 1984).

(4) *The effect of the substance on human health.*

Methionine is essential in small amounts in the human diet, and is sold over-the-counter as a dietary supplement. The L- form of methionine is used extensively in human medicine for a variety of therapeutic purposes, including pH and electrolyte balancing, parenteral nutrition, pharmaceutical adjuvant, and other applications. It is in fact one of the top 800 drugs in human medicine (Mosby, 1997). Methionine may cause nausea, vomiting, dizziness, and irritability and should be used with caution in patients with severe liver disease (Reynolds, 1996).

The D- form of methionine is not well utilized by humans (Lewis and Baker, 1995). Individuals may have allergic reactions to the D- isomers or a racemic mixture of DL-methionine. While a number of amino acids are considered GRAS for human consumption and as feed supplements, DL-methionine is not (see 21 CFR 172, 21 CFR 184, and 21 CFR 570.35). DL-methionine is unique among amino acids cleared for food use in that it is the only one listed that explicitly says it is not for use in infant feed formulas (21 CFR 173.320). When heated to decomposition, methionine emits dangerous and highly toxic fumes (NIEHS, 1999).

(5) *The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.*

Interactions and Imbalances

Protein is required for body development in all growing birds, and layers also need a good proportion since eggs consist of 13-14% protein. Broilers also need high energy diets as they are commericially raised to grow rapidly to reach about 4.4 lbs in 8 week at a desired food conversion rate of 1.8 (consuming less than 8 lbs of feed total) (Sainsbury, 2000). This is a 50-55 fold increase inbody weight by 6 weeks after hatching, which leads to a high amino acid requirement to meet the need for active growth (NRC 1994). The dietary requirement for protein is actually a requirement for the amino acids contained in the dietary protein. Protein quality is related to the proper balance of essential amino acids in the diet. The presence of nonessential amino acids in the diet also reduces the necessity of synthesizing them from the essential amino acids (NRC 1994).

Amino acids in the body are constantly in flux between three states: stored in tissue, oxidized from tissue to free amino acids, and digested and excreted as uric acid. If some nonessential amino acids are low, they may be synthesized from others in the free amino acid pool, or degraded from those stored in tissue. Deficiencies or excesses of a particular amino acids can cause problems in availability of other amino acids (Buttery and D'Mello 1994; Baker 1989). Intact proteins (as in natural grains) are more slowly available in the digestive system, while pure sources of amino acids are more bioavailable than intact-protein sources (Baker, 1989). Excesses of some amino acids in an unbalanced source of crude protein can reduce feed intake and depress amino acid utilization (Pack, 1995). Depressed feed intake and growth at excess intake levels of protein has recently been attributed to insufficient supply of vitamin B-6 which is required to metabolize the sulfur amino acids (Scherer and Baker 2000).

The requirement for sulfur containing amino acids of methionine, cystine, and cysteine can be misjudged due to inaccurate accounting for the availability of cystine in the diet (NRC, 1994).

Other cases have shown significantly higher weights and faster gains from amino acid (lys+met) supplementation (Slominski et al, 1999). Also, the digestibility of practical ingredients, such as corn and soybeans, appears to be on the order of 85% or more (NRC, 1994).

Amino acid requirements may be affected by environmental temperature extremes, basically because of the effect on feed intake, but amino acid supplementation will only affect weight gain if it improves feed intake (Baker 1989; NRC 1994). Interactions between deficiencies of methionine and several vitamins and minerals

have also been documented, and suggest that other dietary factors in addition to total protein have an effect on the efficency of amino acid utilization (Baker, D.H. et.al, 1999).

Environmental Impact

Managing the nitrogen cycle is seen as a challenge to livestock producers (Tamminga and Verstegen, 1992; Tamminga, 1992; Morse, no date). Poultry layer operations are experiencing increased costs and regulations for manure management (Sloan, et al., 1995). Supplementation with amino acids may allow dietary protein and excretory nitrogen levels to be reduced with a minimum reduction in egg output and no loss in weight gain in broilers (Summers, 1993; Sloan et al., 1995, Ferguson, et.al 1998). Excess ammonia build up in poultry houses can be a hazard to workers and birds if not properly ventilated (Ferguson, 1998).

Feeding systems that reduce levels of protein fed using amino acid supplementation are not the only means identified to reduce nitrogen pollution from animal manure. Other potential solutions include lower animal densities; more frequent rotations; better manure storage, handling, and application techniques; use of enzymes; improved processing of the feed; and selection of more appropriate land and locations to graze and shelter animals (Archer and Nicholson, 1992; Tamminga, 1992; Tamminga and Verstegen, 1992). Increased digestiblilty of protein in feeds suplemented with microbial phytase provided better availabilty of most of the amino acids other than lysine and methionine and allowed for reduced P and Ca levels in feed, a goal in reducing phosphorus overload from poultry manure (Sebastian 1997). Another study found that reduced crude protein and energy content were needed in enzyme supplemented broiler diets, although availability of individual amino acids were not improved equally and were still deemed to need balancing (Zanella, et al 1999).

One grower reported success with innovative housing design that allows twice daily cleanout of manure, combined with a commercial composting operation (La Flamme, 2001). Manure from organic operations has potential added value to organic crop farmers seeking to avoid manure from conventional operations. Some markets in the EU require that imported crops are documented to be grown free of "factory farm" manure, requiring additional verification from U.S. certifiers (McElroy, 2001).

Impacts on Bird Health

A number of reports cite a benefit of methionine supplementation on reduced immunologic stress (Klasing, 1988; Tsiagbe et al, 1986). Immunologic stress is considered to be a response to microbial challenges, in these experiements due to injections of E. coli and Salmonella and other pathogens. This causes decreased feed rates and lower rates of growth. Chicks that received deficient levels of methionine were more subject to an impaired immune response. These experiments seem to be more applicable to a high density confinement system or high density production system in terms of bird treatment, and may not be very relevant to an organic system approach.

A problem exacerbated by excess methionine is hepatic lipidosis, a condition of excessive fat in the liver commonly associated with caged birds and is related to the fact that wild diets are much lower in fat than seed diets fed to captive species (Aiello, 1998). This can be mangaged by a well balanced diet, and is reportedly not a problem in free range birds in organic systems (Krengel, 2001). Enteritis is a disease frequently observed in poultry that do not have access to the soil and green growing plants (Titus, 1942). Well managed pasture would prevent this cause of the disease.

Reduced feathering has been reportedly linked to lack of methionine and cystine (Elliott, no date). Many other factors are also involved, including deficiencies of other amino acids, vitamins, zinc, feather pecking in cage systems, and cannibalism (Elliot, NRC 1994). Increased protein level is correlated with reduced feather loss and cannibalism (Ambrosen, 1997).

(6) *The alternatives to using the substance in terms of practices or other available materials.*

Birds raised on pasture with access to insects and worms historically did not need supplementation (Morrison, 1951), and smaller scale pastured operations have success without the need for synthetic supplements (Salatin, 1993). Pasture quality will vary according to field conditions and the season. However, free range poultry on well managed pasture are able to supplement their diets with insects, annelids, and fresh green forage (Smith and Daniel, 1982). The two most limiting amino acids, methionine and lysine, are found in richest sources in proteins of animal origin. Common natural sources of these amino acids have traditionally been fish meal and meat meal, especially for starter chicks and broilers (Sainsbury, 2000). The USDA organic program final rules do

not allow the use of meat meal as feed for poultry or mammals and may or may not allow fish or crab meal (7CFR 205).

Diets can be formulated without supplemented synthetic acids to meet the objective of adequate methionine percentages, but this usually requires an increase in crude protein level of the diet (Hadorn, 2000). Many studies have been done to identify a cost effective method of lowering protein content by supplementing with methionine and lysine. Often the control treatments are non-supplement grain based diets. A comparison study using supplemented and non-supplemented diets found that adequate dietary methionine can be attained, at a cost of higher intake of protein and less protein efficicy ratio (Emmert 2000). Another study fed a control diet using only corn and soy to satisfy amino acid levels compared to reduced protein supplemented with methionine and lysine, and these treatments were considered successful because performance was not lowered in 4-5 experiments (Harms, 1998).

Rice and casein offer potential novel available sources of methionine (Lewis and Bayley, 1995). Yeast protein has long been known as a rich protein source relatively high in methionine+cystine (Erbersdobler, 1973; National Research Council, 1994), as well as phosphorous and B-complex vitamins (Morrison, 1951). As a natural feed supplement, NOSB should advise whether yeast is considered agricultural and required from organic sources or permitted as a natural substance. Other potential sources of available methionine for poultry appear to be sunflower meal and canola meal (Waibel et al., 1998). These natural sources are all currently of limited availability in organic forms. Alfalfa meal is reported to be a good additional protein source, though difficult to blend in commercial formulations. Optimally balancing these nutrients may be challenging to feed processors and livestock producers.

Feed sources with high percentages of methionine are bloodmeal, fish meal, crab meal, corn gluten meal, and sunflower seed meal (National Research Council, 1994). If fish meal were permitted, there is a lack of supply that does not contain ethoxyquin, a synthetic antioxidant not permitted under the final rules. A limited supply of fish meal preserved with natural tocophorols has gone mostly into the pet food market (Mattocks, 2001). Corn gluten and sunflower seed meal are not currently very available in organic form, and feed formulators and nutritionists have reported difficulty in meeting NRC requirements for methionine based on currently available organic plant protein sources (Mattocks, Morrisson, Simmons, 2001). One feed mill operator feels he can meet even broiler needs with a combination of crab meal (at 75 lbs/ton or 3.75%) and organic corn gluten (Martens, 2001). Crab meal is a by-product of crab processing and not treated with preservatives but has limitations due to salt content. Another certified feed mill produces layer and range broiler rations without synthetic amino acids based only on plant products, including corn, soy, barly, oats, wheat, field peas, and flaxmeal. These products are labeled at a minimum of 0.3% met and 0.6% lysine, but reportedly achieve good results (White 2001, VOG).

NRC requirements for amino acids and protein are designed to support maximimum growth and production. The recommended levels for methionine in poultry depend on species, stage, and level of feed consumption. For chickens, recommendations for layers range from 0.25% to 0.38% and for broilers 0.32 - 0.50%. NRC notes that maximum growth and production may not always ensure maximum economic returns when protein prices are high, and that if decreased performance can be tolerated, dietary concentrations of amino acids may be reduced somewhat to maximize economic returns (NRC, 1994). Methionine is known to have a direct effect on egg weight (size) and rate of lay, and is used by some producers to manipulate egg production to meet market needs, such as to increase egg size in younger birds, reduce it in older birds, or produce more eggs in off peak market periods (NRC 1994; Harms 1998; Simmons 2001). A reduction in rate of gain in broilers (longer time to finish) would be an outcome of lower than optimal methionine levels. Unless the diet contained other forms of sulfur containing amino acids (cystine or cysteine), problems with inadequate feathering might be encountered (Simmons, 2001).

Temporarily confined poultry can be fed a practical organic corn / soybean ration. Depending on market conditions and on how other parts of the standards evolve, novel organic products can be developed as supplements. Among the potential alternative sources include organic dairy products such as casein (National Research Council, 1982 and 1994).

Macroorganisms commonly found in healthy pasture soils cannot be discounted as a source of nutrient cycling in free-range poultry systems. Given the natural feeding habits of poultry and other birds, the use of earthworms is a logical source of protein in chicken feed (Fisher, 1988). Earthworm populations in a pasture depends on a

number of factors (Curry, 1998). The amino acid content of earthworms will vary depending on species and food source. However, earthworms have been found to accumulate and concentrate methionine found in the ecosystem in proportions greater than for other amino acids (Pokarzhevskii, et al., 1997). As a feed supplement, earthworms have been found to equal or surpass fish meal and meat meal as an animal protein source for poultry (Harwood and Sabine, 1978; Toboga, 1980; Mekada et al., 1979; and Jin-you et al., 1982 all cited in Edwards, 1998).

Earthworms can play a role in moderating nitrogen losses as well. Enzyme treatment of feedstuffs can improve amino acid availability and also reduce nitrogen pollution (Tamminga and Verstegen, 1992), as can changes in stocking density, rotations, and manure handling.

(7) *Its compatibility with a system of sustainable agriculture.*
In 1994 the NOSB recommended that feed and feed supplements be produced organically. When considering the review of feed additive vitamins and minerals, an NOSB statement of principles advised that non-synthetic viamins and mineral sources are preferable when available. The NOSB also advised that a farm plan should reflect attempts to decrease or eliminate use of feed additives when possible (NOSB, 1995).

A constraint to optimal production in modern organic systems that are not able to utilize pasture based systems appears to be adequate organic sources of the first limiting amino acid, methionine. The allowance of isolated amino acids facilitates the use of the lowest cost, non-diverse corn-soy ration. It is the basis of conventional confinement animal production systems which may be considered as antithetical to the principals of organic livestock production. The source and method of production of synthetic amino from non-renewable fossil fuels and toxic chemicals is also questionable in compatiblilty with system of sustainable agriculture.

The use of synthetic amino acids increases animal production by increased efficiency of protein conversion, which lowers feeding costs and reduces nitrogen content of the waste output. While this is not by itself unsustainable, synthetic amino acids discourage the integration of a whole-systems approach to cycling nutrients, particularly nitrogen, as part of an integrated crop-livestock production system. Allowance of synthetic sources of amino acids may discourage market development of organic plant sources, such as seed meals.

Increased efficiency of protein conversion reduces the amount of nitrogen excreted (Summers, 1993; deLange, 1993). The cycling of nutrients from animals is part of an integrated farming system, and the environmental effects of manure management requires looking at the big picture (Archer and Nicholson, 1992). What is viewed as a liability in confinement animal systems—nitrogen production—is seen in cropping systems as a limiting factor resource. Reduction of nitrogen pollution may require improved range or pasture management, and with that either more frequent rotations or lower stocking rates.

TAP Reviewer Discussion[2]

Reviewer 1 *[Eastern –Ph.D. Senior Research Chemist, reviewer in organic certification agency]*

Commercially available amounts of Methionine can only be produced synthetically. All current routes are modification of the Strecker synthesis, and they share the same raw materials: acrolein, methanethiol (methyl mercaptan), along with various sources of ammonia and cyanide (McPherson, 1966; Gerhartz, 1985). The fundamental raw materials are hydrocarbons, sulfur, inorganic salts, and Nitrogen (McPherson, 1966).

Methionine (Me) fed to livestock is a synthetic source of non-protein Nitrogen. Being an amino acid, it can be directly utilized in protein synthesis and, in effect, substitutes for protein derived from foodstuff. Me is metabolically linked with cystine and choline and is necessary for producing keratins used in feather growth (NAS Nutrition Req. of Poultry, 1994; Elliot no date). It is also considered the main limiting amino acid in modern poultry diets (NAS Nutrition Req. of Poultry, 1994). Me deficiency has become a problem in recent years due to the increasing use of low cost soybean protein and bird genetic selection for increase broiler growth or egg laying ability. High protein

[2] *OMRI's information is enclosed is square brackets in italics. Where a reviewer corrected a technical point (e.g., the word should be "intravenous" rather than "subcutaneous"), these corrections were made in this document and are not listed here in the Reviewer Comments. The rest of the TAP Reviewer's comments are edited for any identifying comments, redundant statements, and typographical errors. Text removed is identified by ellipses […].Additions to the TAP review text were incorporated into the review. Statements expressed by reviewers are their own and do not reflect the opinions of any other individual or organizations.*

diets are necessary for fast growth but low feed cost are needed in business plans seeking to increase market share for Organic poultry products. In order to address the need for synthetic Me in Organic poultry management, several questions need to be considered:

- Is synthetic Me necessary to grow healthy poultry?
- Are there food-based protein that can economically provide balanced protein (including Me), maintaining both bird health and producer economic viability?
- Is a management system that relies on a critical synthetic feed additive consistent with the principles of Organic Agriculture?

The increasing need for Me is founded in the simplification of poultry diet to corn+ soybean + minerals that started in the 1950's (Baker, 1989). Corn is one of the best energy sources of the grains (Wright 1987). However, its protein level is low (~ 8%) and a corn based diet is low in Lysine (Wright 1987). Soybean is high in protein and is an economic source of Lysine. It is low in Me, however, and diets that use soybean to increase protein (for broiler growth or more eggs) can cause Me to become the limiting amino acid (Garcia, Pesti, and Bakalli 99; Waibel et. al. 1999).

The low concentration of Me in high-protein corn/soybean feed mixes has lead to wide use of synthetic Methionine supplementation in poultry feed mixes. Methionine is found in many feed mixes (both Organic and traditional) as an individual component and also contained in vitamin/mineral packs. Many poultry rations contain Me in both forms. Both the NAS and the Merck Veterinary Guide (8'ed 1998) set around 0.3 wt. % of total ration, which is higher than available in the average corn/soybean mix.

The NAS recommended Me level is set for maximum performance. The report states "The protein and amino acid concentrations prescribed as requirements herein are intended to support maximum growth and production" (NAS Nutrition Req. of Poultry, 1994 p. 10). The NAS report and the studies supporting Me supplementation reviewed primarily use growth rate to determine proper Me levels in the diet (either total weight or percentage of breast meat), (Tsiagbe et. al 1987; Edwards and Baker, 1999; Garcia et al 99, Waibel et al 1999; Degussa Feedback Nov 1995 and March 1996). Reduced growth rate can be a symptom of amino acid deficiency (D'Mello). However, this type of growth retardation is accompanied by other physiological changes that take place to compensate for the deficiency. These changes can be hematologically measured (Torun 1979). In true deficiency, total body nitrogen balance would also go negative (Wintrobe et. al. 1970). The above reports do not present evidence clearly showing that the unsupplemented slow growing birds studied are malnourished.

Lower than desired growth (or egg production) can also be due to less than optimal protein utilization in high protein rations. This is an economic consideration, not a health one.

True deficiency in sulfur-bearing amino acids can cause improper feathering (Elliot, no date). Elliot notes that Me supplementation can improve feathering. The paper does not give a minimum Me level, or describe how feathering is effected by the interactions of Methionine, Cystine, and Choline. Tsiagbe et. al 1987 b note that the immune response of chicks challenged with an antigen changes with supplemental Me. Me was observed to activate certain parts of the chick's immune system. The mechanism was unclear and the therapeutic effectiveness of Me supplementation was not studied. Enhancing poultry immune response to infectious diseases could be an important benefit of ration based Me; especially as aid in fighting Newcastle's Disease. The data seen so far, however, is equivocal. Therefore, this reviewer must conclude that the primary use of crystalline Me in the current petition is to balance corn/soybean rations for maximum feed utilization and poultry productivity.

As related in the body of this TAP review, pastured-based poultry can adequately maintain a proper diet (Salatin, 1993). Before the widespread availability of crystalline amino acid supplements, livestock nutritionists recommended mixing different types of food stuffs to achieve a supplementary relationship between the different proteins (Maynard and Loosli, 1962; Hart and Steenbock, 1919). This system used a combination of grains, roughage such as alfalfa, and a source of high quality protein such as gluten meal, whey, milk, or tankage. Hart and Steenbock found that swine fed corn + milk has initial better weight gains and produced less feces that swine fed corn + milk + alfalfa. The absence of roughage, however, led to long term deleterious health effects and shortened life span.

Dairy products (whey or powdered milk) contain good quantities of Me. Fresh and powdered organic cow's and goat are both available. Their availability should increase as more organic processed food products appear on the market.

Corn Gluten Feed (CGF) is also available and a good source of Me (crude protein usually 21 %). CGF has intermediate metabolizable energy (ME) and is low in lysine, tryptophane, and Calcium (Wright, 1987). It can successfully substitute for part of other high protein ration components (Wright, 1957). Corn gluten meal is a much better protein source. It is 60% protein, high Me, and is a good source of Me and cystine (Wright 87). It is low in lysine and tryptophane but is complemented by the amino acid balance in soybean.

Several other plant-based protein sources are discussed in the main body of this TAP review. All plant-based poultry rations with Me levels of 0.3% are currently on the market and have been shown to produce 4 lb. broilers in eighth weeks (White, 2001). Animal-based protein sources are also available. Animal-based protein is consistent with the specific needs of chickens and turkeys; both being natural omnivores. Crab meal is an excellent balanced protein source that is close to the birds' natural prey. Crab meal needs to be balanced with other feed ingredients to minimize salt in the diet and to keep the crab meal and mineral supplements within the 5% nonorganic ingredient limit (local certification agency requirements) (Martens, 2001).

Rations that supply adequate Me for maximum poultry growth and egg production appear to exist. Some of these diets will be initially more expensive than the corn/soybean + crystalline methionine ration. The production and distribution systems for Organic feed corn and soybeans are established and large scale. Except for crab meal and corn gluten meal that are currently being used in poultry feed, the other soybean substitutes are mostly being sold into other markets. As the poultry market develops for these rations, prices will certainly fall.

Some producers have argued that current bird genetics are optimized for corn/soybean rations, and that production would suffer if other protein sources were substituted for part of the soybeans. Producers of pastured ruminates and poultry are facing and solving similar problems. Older breeds of poultry still exist and people such as Tom Shelly of Virginia are actively working to breed back older traits into over-specialized poultry. Companies like Tysons also have active programs of collecting poultry genetics from around the world and this type of information could be used to guide breeding work. The switch from soybean + Me to soybean + other high Me protein source is not too large for current poultry genetics to handle, but if genetics need to be fine-tuned again, stock and breeders are available.

Methionine appears to present no human health problem. Crystalline Me is well utilized by poultry (Baker 1989) and risk to humans from poultry products or excretions is minimal (Kleeman et al 1985). Excreted Me also appears to pose no environmental problems. It is rapidly photo-degraded in surface water, and is readily metabolized by bacteria in sediments (Kiene and Visscher, 1987).

Several methionine feedstocks, however, present both environmental and human health difficulties. Hydrogen Cyanide is a poisonous, flammable gas that is unstable in the presence of moisture. The low boiling and flash points make Hydrogen Cyanide especially hazardous [25.7 ° C. and -17.8° C. respectively] (Klenk et. al. 1987). Acrolein is a flammable and toxic liquid. It is highly volatile (flash point - 26° C.) and can explosively polymerize if exposed to many contaminants (Ohara et. al. 1987).

Synthetic amino acid supplementation can lower nitrogen emissions of poultry excreta by reducing the crude protein (food) in the diet (Ferguson, 1998). Synthetic amino acids are more absorbable than protein bound amino acids and can be balanced to minimize the amount of amount of protein catabolized to correct for a limiting amino acid. Careful control of temperature and humidity can also be used to further reduce nitrogen loss due to metabolic activity to maintain body temperature.

Reduction in bird nitrogen emissions has been argued to decrease ventilation energy usage. Less energy used to ventilate poultry houses may be real (no data given). However, is the energy used to product the quantity (feed + synthetic AA + house energy) more or less than that needed to produce (more feed + house energy)? Each amino acid needs different amounts of energy (in fossil fuel equivalents FFE). For example, the Me feedstock Hydrogen cyanide requires reaction temperatures of 1300° to 1500° C. during production ((Klenk et. al. 1987). The reviewer can not estimate whether savings in ventilating poultry houses is greater than the energy used to make the synthetic amino acids in the feed. A few years ago, the Green plastics industry touted that plastics made from agricultural

products used less FFE than plastics derived from petrochemicals. Detailed and conservative calculations showed the opposite to be true (Gerngross, 1999).

Simple Me supplementation will have no major effect on amount of manure birds produce or on global nitrogen cycling. Another amino acid will become the prime limiting and the food-based protein will be inefficiently utilized. The idea of a synthetic diet, however, puts Me into a larger context. Food utilization is inefficient. Individual organisms in an ecosystem are very poor utilizes of resources. Animals leave many nutrients behind as manure, seed is a small proportion of plants biomass, and large batch microbial fermentation creates so much heat that the organisms would die unless cooled by an outside energy source. Ecosystem (or agro-ecosystem) efficiency can only be seen when the whole system is considered. One organism's waste is another one's food.

The American Organic Standards principles of Organic Production and Handling state that "Organic agriculture is based on holistic production management systems which promote and enhance agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. Organic agriculture emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. These goals are met, where possible, through the use of cultural, biological, and mechanical methods, as opposed to using synthetic materials, to fulfill specific functions within the system." (AOS standards Oct 1999).

A synthetic diet is an attempt to isolate a part of an agro-ecosystem and then fine tune this part into efficiency. This process requires external inputs. The use of an amino acid supplement like Me to increase feed utilization efficiency is food substitution. Fundamentally, there are many similarities between amino acid substitution by synthetic Me and nitrogen substitution by feeding synthetic non-protein NPK compounds like Urea (NAS, 1976; Featherston, 1967). Feeding urea to livestock is prohibited (7 CFR Part 250.237.b.4).

The evidence presented to this reviewer indicates that synthetic Methionine supplementation is currently used to increase feed utilization. Poultry rations composed of natural food stuffs can give adequate Me levels in both broilers and layers. Methionine should therefor be considered a prohibited synthetic substance.

If the NOSB decides to prohibit Methionine poultry supplements, this reviewer would request that a phase-out period be established to allow producers to find different protein sources (to enhance soybeans) without disrupting their own operations or the current markets for the alternative protein sources. I would also request that the petitioners for Me supplements further research the possible immune enhancing effect of Me. If it is efficacious against infectious poultry diseases, I would hope that they would re-petition for that [medical] use.

[Conclusion:]
Methionine should be prohibited. Suitable high protein feed sources are available. Methionine supplementation is primarily to increase growth and production, not to maintain bird health.

Reviewer 2 [*Midwest--veterinarian performing product research and development, including organic feed formulation*]

[Agrees that the database is accurate, and agrees with the evaluation of OFPA criteria with the following comments.]

[Criteria 3 -The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems;]
I agree with the criteria evaluation, and add the following: Methionine is widely used in the general poultry ration business. Its rates of inclusion are well understood and unlikely to be misused, misformulated, or cause detrimental interactions or results.

[Criteria 5 - The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility in the soil), crops and livestock]
I agree with the criteria evaluation with the following comments: The requirement of sulfur containing amino acids can also be misjudged due to outdated tables showing the typical nutrient content of feeds. This is especially true for the recent past corresponding to the increase in synthetic sulfur containing amino acids. The Clean Air Act provisions reducing atmospheric sulfur levels and minimal applications of sulfur containing fertilizers lead to less than optimal sulfur supplies needed for plants to make sulfur containing amino acids.

[Criteria 6 - The alternatives to using the substance in terms of practices or other available materials]

I agree with the criteria evaluation with the following comments:

Lower than predicted levels of sulfur containing amino acids in plants grown with lower than optimal levels of soil sulfur means that pastured animals may no longer be able to meet their requirements as once thought.

Pasturing is recognized as not feasible in many temperate climates on a year around basis. The short life span of broilers and cyclic nature of laying hens means meeting these animals' nutritional requirement strictly through access to pasture is impossible for some poultry producers.

Alternative animal protein sources are readily available in the feed industry. The suitability of these sources for organic production is of question. Much fish, crab, and shrimp meal result from harvesting natural populations of these animals. Are these natural supplies considered organic based on current knowledge of oceanic pollution? Organic aquaculture is not a large enough industry to support the needs of the poultry feed industry, nor does it appear that it will be for quite some time, if ever. The antioxidant additives used on high oil fish meal (menhaden, anchovy, etc.) are also questioned for organic suitability.

The decision may come down to 'the lesser of two evils'. Should synthetic amino acids be allowed or should natural proteins with some questions be allowed? Either way, they should be restricted to those species that specifically need them, more specifically poultry.

[Criteria 7 - Its compatibility with a system of sustainable agriculture]
I agree with the criteria evaluation with the following comments: Synthetic amino acid use discourages diversity of animal feed sources (e.g. typical corn - soybean diet).

[*Discussion]*
The letter [Kringel, 2001] overstates the hardships of ration diversity for poultry rations. Sources such as barley, wheat, and ground alfalfa are used in rations in my area. The alfalfa is only difficult for milling operations to mix. On farm mixers have no problem with it.

My experience in poultry rations is that some source of methionine is necessary for acceptable growth, egg production, and health. The use of synthetic methionine appears to be geared toward maximizing production in the majority of cases. The primary reason that most feed companies utilize it is that it allows them to use only corn and soybeans as grain sources in cheaper rations. This means fewer bins, large supplies of cheap grain, and easier blending.

Birds will survive with lower than NRC levels of methionine, but they will grow slower, produce less eggs, and have some disease problems such as pecking disorders.

Yeast is not a feasible option at this time due to availability. There are also some questions regarding the GM status of grains used in producing several types of yeast products suitable for animal feeding, primarily in the brewers and distillers yeast products.

Limiting the synthetic methionine included in rations to 0.1% would help alleviate my concern of limited ration diversity. Philosophically there may be some question. If it is good enough to use 0.1%, why not the full rate? Even so, I am in favor of the 0.1% level maximum as an annotation.

Genetics for modern lines of birds does seem to have a bearing on methionine requirement according to some poultry gurus I have talked to in the past. They say it is true of laying hens as well.

[Conclusion – Summarize why it should be allowed or prohibited for use in organic systems.]
The only valid reason I can find for this material to be allowed is that no good alternatives exist that meet current organic standards. The merits of the material itself would indicate that it should be prohibited. One possible compromise may be to place the material in the restricted use category similar to synthetic minerals and vitamins. Restriction annotations could include "only when alternative sources, such as diverse protein source ingredients or suitable animal proteins (e.g. fish, crab, shrimp, whey, etc.) are not available." This annotation leaves a big loophole. Feed producers can easily say that no alternatives are available, and who is going to subjectively decide what alternatives are suitable or adequate nutritionally?

A second alternative is to list a synthetic methionine use level restriction of 0.1% of the ration. This can be avoided by feed manufacturers simply by saying that a grain blend is to be used with some other feed source, such as pasture. Maybe a better restriction is 0.1% maximum in any feed or blend of feed used for poultry.

Another alternative is to table the decision on synthetic amino acids in general and immediately evaluate the aquatic sources of natural sulfur containing amino acids. A decision would have to be made as to what may be acceptable for these sources and whether these sources are more acceptable than the synthetic amino acids or less acceptable. One of these two materials appears to be necessary to raise poultry under the conditions encountered by many producers.

[*Additional Comments*]
My personal recommendation is that the natural aquatic animal sources are the better method for obtaining sulfur containing amino acids. I think that natural populations of aquatic life should be acceptable to the organic feed industry. Farm raised aquaculture should meet organic production standards for inclusion into organic rations.

After reading all the comments from various sources included in the information packet, I think the only real issue for aquatic sources is that of the antioxidants. One issue is that ethoxyquin is sometimes not listed on the label of some fish meal sources, so ration formulators may not even know that it is in the fish meal.

Cost of ingredients should never be a factor in determining their suitability for organic production.

The sources are quite available in all locations.

Salt content limiting crab meal inclusion rates can be worked around if the meal is combined in rations containing diverse ground feed sources – corn, soybean, wheat, barley, oat, sunflower, pea, alfalfa or clover, etc. – even for birds that do not have access to pasture. A 0.1% maximum inclusion rate of synthetic methionine would also help with the limitations caused by the salt content of crab meal.

I am in favor of allowing these aquatic sources, even if only for poultry rations, instead of synthetic amino acids.

The last issue I will raise, is the effect of this material's status on chelated trace mineral's possible status in organic production systems. Commercially available chelated trace minerals are primarily bound to proteins, peptides or individual amino acids.

[Recommendation Advised to the NOSB:]
[(a) The material is:] Synthetic
[(b) For Crops and Livestock, the substance should be:] Added to the National List.
[(c) Suggested Annotation, including justification:] Enzyme digested natural protein sources preferred, source must be non GM. For poultry rations only. Only when alternatives are not available including ration diversity and acceptable animal and plant sources. Not to exceed 0.1% by weight in any feed source directly fed to poultry.

Reviewer 3 [*Western-Midwestern veterinarian providing technical services to ranchers]*
Methionine is an amino acid, a necessary component of certain proteins needed by livestock. L-methionine is a synthetic amino acid, produced by various chemical methods.

The bottom line is that synthetic amino acids are prohibited in the national rule. They are also prohibited internationally. Feeding of synthetic amino acids is neither compatible with a system of sustainable agriculture nor does it follow the principles of organic agriculture. Both of these systems are based on a premise of access to the natural environment, with less reliance on off-farm inputs and better management of the ecosystem. This premise precludes the use of synthetics generally.

According to Dr. Jason Emmert, poultry nutrition professor with the University of Arkansas, poultry producers are able to supply methionine in natural feedstuffs and pasture access, without the need for supplemental methionine. Pasture access will provide fresh grass and animal protein in the form of insects and earthworms. Grain rations will have to have additional soybean meal added, bringing the protein level to 23-24% crude protein. These diets can be

provided to poultry of all ages. Thus, many of the points regarding level of methionine recommended or age of bird needing additional supplementation are irrelevant to this discussion.

One issue brought up for the need to supply synthetic methionine is the nitrogen level of litter. Nitrogen is often the limiting factor in farming systems, especially organic farms. The availability of organic litter could be another product of organic poultry operations, adding diversity and income to the farm. It could also help the farm to diversify by using the litter as fertilizer for pasture or other crops.

In rereading the article about immunological stress, methionine deficient diets actually had less of a reduction in growth rate and feed efficiency in pathogen stressed chicks than methionine sufficient diets. [...] Too much of this research is done in strict confinement settings, with maximum growth rate in minimum time. Trying to apply research done in this production system to a sustainable (access to pasture, the outdoors, more natural feedstuffs, etc.) system can only be done to a certain extent.

There is the concern that the poultry genetics are limited. This concern is valid, but like many of the other concerns listed, such as the need for more organic sources of various feedstuffs, the new organic rule will give the opportunity for supporting businesses to be formed to supply the needed genetics, feeds, and other allowed products.

The supporting documentation and research for this TAP review indicates the great need for research investigating the unique management needs of organic farming systems. The articles referenced are about research geared for the confinement poultry industry whose goal is to produce maximum sellable product for the least amount of money in the least amount of time with little regard to the sustainability of this production system. I also feel that if we make an exception to the no synthetic amino acids ruling, we will hurt the public image of organic farming (quicker than it may happen anyway) and we also open the door to other exceptions. I feel it important to retain the "no" ruling now. It can be changed in the future if further research aimed at organic farms and their management strategies shows that adding a small amount of synthetics does indeed improve the life, health, and overall production of the organic chicken.

In summary, the references and accompanying materials cover the points for continuing to prohibit synthetic methionine or any other amino acid. Methionine can be supplied through natural products, in quantities sufficient to meet birds' nutrient requirements, at any age. This eliminates any necessity for synthetic methionine.

Conclusion

All of the reviewers agree that the forms of methionine unders consideration, DL methionine, L methionine, and the hydroxy anlaog described in this review are synthetic. Two of the three reviewers find their use to be incompatible with organic systems and recommend prohibition. The third finds that use should be allowed only when natural sources or dietary sources are unavailable, and feels they should be allowed due to the lack of alternatives that meet organic standards. All reviewers agree that an allowance for synthetic amino acid use discourages diversity of animal feed sources, and that natural intact protein sources are more compatible with organic principles.

By substituting a non-renewable synthetic input for organically grown crops as a source for nutrition, synthetic amino acids would thus reduce the amount of acreage, and the market created for organic feed and forage crops. Farmers who seek organic certification have less of an incentive to provide clean, fresh pasture as a primary source of animal nutrition if they have available synthetic amino acids. There is also some confusion in the livestock industry as to the organic status of natural alternatives, such as yeast, casein, and fish or crab meals. These alternatives are more in keeping with the NOSB recommendations for natural feed supplements than those from synthetic or potential GMO sources. A requirement for natural, non-GMO sources of methionine, and a clarification of the status of some of these alternatives will stimulate market development in organic and approved feedstuffs.

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*Note: * = included in packet compiled for NOSB*

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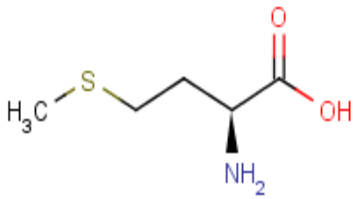
Hazardous Substances Data Bank – L-Methionine

L)-Methionine

CASRN: 63-68-3



Environmental Fate/Exposure Summary :
(**L)-Methionine's** production and use as a nutritional supplement in animal feeds may result in its release to the environment through various waste streams. (**L)-Methionine** is formed in natural waters through metabolism of naturally occurring proteins and is a naturally occurring amino acid, being present in foods such as maize, rice, wheat, potato, soybeans, lettuce, apples, oranges, beef, fish, milk, cheese and eggs. If released to air, an estimated vapor pressure of 8.1X10-8 mm Hg at 25 deg C indicates (**L)-methionine** will exist in both the vapor and particulate phases in the atmosphere. Vapor-phase (**L)-methionine** will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 7.5 hours. Particulate-phase (**L)-methionine** will be removed from the atmosphere by wet or dry deposition. (**L)-Methionine** does not contain chromophores that absorb at wavelengths >290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight. If released to soil, (**L)-methionine** is expected to have very high mobility based upon an estimated Koc of 8. The pKa values of (**L)-methionine** are 2.28 and 9.21, which indicate that this compound will exist as a zwitterion which may affect its adsorption to soils and sediments. Volatilization from moist soil is not expected because ions do not volatilize. (**L)-Methionine** may not volatilize from dry soil surfaces based upon its vapor pressure. Using a laboratory activated sludge system, (**L)-methionine** exhibited an 80% theoretical BOD reduction in 16 days, producing 3-mercaptopropionate, methanethiol and dimethylsulfide; this suggests that biodegradation may be an important environmental fate process. If released into water, (**L)-methionine** is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. The pKa values of 2.28 and 9.21 indicate (**L)-methionine** will exist as a zwitterion at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyze under environmental conditions. (**L)-Methionine** has been shown to degrade in sunlit natural water through photo-sensitized oxidation involving singlet oxygen. Occupational exposure to (**L)-methionine** may occur through inhalation and dermal contact with this compound at workplaces where (**L)-methionine** is produced or used. Monitoring data indicate that the general population may be exposed to (**L)-methionine** via ingestion of food. (SRC)
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Probable Routes of Human Exposure :
NIOSH (NOES Survey 1981-1983) has statistically estimated that 28,250 workers (17,038 of these were female) were potentially exposed to (**L)-methionine** in the US(1). Occupational exposure to (**L)-methionine** may occur through inhalation and dermal contact with this compound at workplaces where (**L)-methionine** is produced or used. Monitoring and use data indicate that the general population may be exposed to (**L)-methionine** via ingestion of food or other consumer products containing (**L)-methionine(**SRC).
[(1) NIOSH; NOES. National Occupational Exposure Survey conducted from 1981-1983. Estimated numbers of employees potentially exposed to specific agents by 2-digit standard industrial classification (SIC). Available from, as of Feb 19, 2010: http://www.cdc.gov/noes/ **PEER REVIEWED**

Non-Human Toxicity Excerpts :
/ALTERNATIVE and IN VITRO TESTS/ **L-methionine (**Met) is hepatotoxic at high concentrations. Because Met toxicity in freshly isolated mouse hepatocytes is gender-dependent, the goal of this study was to assess the roles of Met accumulation and metabolism in the increased sensitivity of male hepatocytes to Met toxicity compared with female hepatocytes. Male hepatocytes incubated with Met (30 mM) at 37 degrees C exhibited higher levels of intracellular Met at 0.5, 1.0, and 1.5 h, respectively, compared to female hepatocytes. Conversely, female hepatocytes had higher levels of S-adenosyl-**L-methionine** compared to male hepatocytes. Female hepatocytes also exhibited higher **L-methionine-**L-sulfoxide levels relative to control hepatocytes, whereas the increases in **L-methionine-**D-sulfoxide (Met-D-O) levels were similar in hepatocytes of both genders. Addition of aminooxyacetic acid (AOAA), an inhibitor of Met transamination, significantly increased Met levels at 1.5 h and increased Met-d-O levels at 1.0 and 1.5 h only in Met-exposed male hepatocytes. No gender differences in cytosolic Met transamination activity by glutamine transaminase K were detected. However, female mouse liver cytosol exhibited higher methionine-dl-sulfoxide (MetO) reductase activity than male mouse liver cytosol at low (0.25 and 0.5 mM) MetO concentrations. Collectively, these results suggest that increased cellular Met accumulation, decreased Met transmethylation, and increased Met and MetO transamination in male mouse hepatocytes may be contributing to the higher sensitivity of the male mouse hepatocytes to Met toxicity in comparison with female mouse hepatocytes.
[Dever JT et al; Toxicol Appl Pharmacol 236 (3): 358-65 (2009). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=19236888 **PEER REVIEWED**

Environmental Fate :
TERRESTRIAL FATE: Based on a classification scheme(1), an estimated Koc value of 8(SRC), determined from a log Kow of -1.87(2) and a regression-derived equation(3), indicates that (**L)-methionine** is expected to have very high mobility in soil(SRC). The pKa values of (**L)-methionine** are 2.28 and 9.21(4), indicate that this compound will exist as a zwitterion which may affect its adsorption to soils and sediments(SRC). Volatilization from moist soil is not expected because ions do not volatilize(SRC). (**L)-Methionine** is not expected to volatilize from dry soil surfaces(SRC) based upon an estimated vapor pressure of 8.1X10-8 mm Hg at 25 deg

C(SRC), determined from a fragment constant method(5). Using a laboratory activated sludge system, (**L)-methionine** exhibited an 80% theoretical BOD reduction in 16 days, producing 3-mercaptopropionate, methanethiol and dimethylsulfide(6); this suggests that biodegradation may be an important environmental fate process in soil(SRC).
[(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 15 (1995) (3) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm as of Feb 19, 2010. (4) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007. (5) Lyman WJ; p. 31 in Environmental Exposure From Chemicals Vol I, Neely WB, Blau GE, eds, Boca Raton, FL: CRC Press (1985) (6) Engelbrecht RS, McKinney RE; Sew Indust Wastes 29: 1350-62 (1957)] **PEER REVIEWED**

Environmental Biodegradation :
ANAEROBIC: The biotransformation of (**L)-methionine** in anoxic sediment slurries was found to produce 3-mercaptopropionate(1). In salt marsh sediment slurries, microbial metabolism of (**L)-methionine** yielded methanethiol as the major volatile organosulfur product, with the formation of lesser amounts of dimethylsulfide(2); the decomposition of (**L)-methionine** occurred rapidly in anoxic salt marsh sediments(2). (**L)-Methionine** was highly bioconvertible (68-90% CO2 evolution) in 35-78 day anaerobic degradation studies using waste activated sludge from the San Jose-Santa Clara Water Pollution Control Plant(3).
[(1) Kiene RP, Taylor BF; Nature 332: 148-50 (1988) (2) Kiene RP, Visscher PT; Appl Environ Microbiol 53: 2426-34 (1987) (3) Stuckey DC, McCarty PL; Water Res 18: 1343-53 (1984)] **PEER REVIEWED**

Soil Adsorption/Mobility :
The Koc of (**L)-methionine** is estimated as 8(SRC), using a log Kow of -1.87(1) and a regression-derived equation(2). According to a classification scheme(3), this estimated Koc value suggests that (**L)-methionine** is expected to have very mobility in soil. The pKa values of (**L)-methionine** are 2.28 and 9.21(4), indicate that this compound will exist as a zwitterion which may affect its adsorption to soils and sediments(SRC). One study found that (**L)-methionine** was one of many amino acids that sorbed to carbonate sediments in seawater(5); a positive correlation between surface area (of the sediment) and the amount of sorbed amino acids indicated that sorption from solution (partitioning from the water column to sediment) was a likely mechanism(5).
[(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 15 (1995) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm as of Feb 19, 2010. (3) Swann RL et al; Res Rev 85: 17-28 (1983) (4) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007. (5) Muller PJ, Suess E; Geochim Casmochim Acta 41: 941-9 (1977)] **PEER REVIEWED**

Methods of Manufacturing :
The production method of choice for **L-methionine** is still the enzymatic resolution of racemic N-acetyl-methionine using acylase from Aspergillus oryzae. The production is carried out in a continuously operated fixed-bed or enzyme membrane reactor. Alternatively, **L-methionine** may be produced by microbial conversion of the corresponding 5-substituted hydantoin. With growing cells of Pseudomonas sp. strain NS671, D,L-5-(2-methylthioethyl)hydantoin was converted to **L-methionine;** a final concentration of 34 g/L and a molar yield of 93% have been obtained.
[Eggersdorfer M et al; Ullmann's Encyclopedia of Industrial Chemistry 7th ed. (2008). New York, NY: John Wiley & Sons; Vitamins. Online Posting Date: June 15, 2000] **PEER REVIEWED**

Methods of Manufacturing :
The most economic way for production of D,**L-methionine** is the chemical process based on acrolein, methyl mercaptan, hydrogen cyanide, and ammonium carbonate. beta-Methylthiopropionaldehyde, formed by addition of methyl mercaptan to acrolein, is the intermediate that reacts with hydrogen cyanide to give alpha-hydroxy-gamma-methylthiobutyronitrile. Treatment with ammonium carbonate leads to 5-(beta-methylthioethyl)hydantoin that is saponified by potassium carbonate giving D,**L-methionine** in up to 95% yield, calculated on acrolein. /D,**L-Methionine/**
[Eggersdorfer M et al; Ullmann's Encyclopedia of Industrial Chemistry 7th ed. (2008). New York, NY: John Wiley & Sons; Vitamins. Online Posting Date: June 15, 2000] **PEER REVIEWED**

Interactions :
The use of nitrofurans as veterinary drugs has been banned in the EU since 1993 due to doubts on the safety of the protein-bound residues of these drugs in edible products. Furazolidone (FUZ) is a nitrofuran drug, which has been used for many years as an antibacterial drug in veterinary practice. ... Forty female Sprague-Dawley rats were divided into four groups included the untreated control group; a group treated with FUZ (300 mg/kg bw); a group treated with a mixture of L-cysteine (300 mg/kg b.w.) and **L-methionine (**42.8 mg/kg b.w.) and a group treated with FUZ plus the mixture of L-cysteine and **L-methionine** for 10 days. The results indicated that FUZ induced hormonal disturbances involving thyroid, ovarian and adrenal hormones. Moreover, FUZ increased the micronucleus formation and induced changes in polymorphic band patterns. The combined treatment with FUZ and the mixture of L-cysteine and **L-methionine** succeeded to prevent or diminish the endocrine disturbance and the clastogenic effects of FUZ...
[Ahmed HH et al; Toxicology 243 (1-2): 31-42 (2008). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=17964703 **PEER REVIEWED**

Natural Pollution Sources :
(**L)-Methionine** is formed in natural waters through metabolism of naturally occurring proteins(1). It is one of the nine indispensable amino acids that cannot be synthesized to meet body needs in animals and therefore must be provided in the diet(2). (**L)-Methionine** is a

naturally occurring amino acid constituent of egg albumin, casein, and beta-lactoglobulin proteins(3); (**L)-methionine** occurs naturally in foods such as maize, rice, wheat, potato, soybeans, lettuce, bean, tomato, apples, oranges, beef, veal, fish, milk, cheese and eggs(3).
[(1) Kiene RP, Visscher PT; Appl Environ Microbiol 53: 2426-34 (1987) (2) NAS, Food and Nutrition Board, Institute of Medicine; Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press, Washington, D.C. (2005). Available from, as of August 17, 2010: http://books.nap.edu/openbook.php?record_id=10490&page=589 (3) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007.] **PEER REVIEWED**

Environmental Fate :
AQUATIC FATE: Based on a classification scheme(1), an estimated Koc value of 8(SRC), determined from a log Kow of -1.87(2) and a regression-derived equation(3), indicates that (**L)-methionine** is not expected to adsorb to suspended solids and sediment(SRC). The pKa values of 2.28 and 9.21(4) indicates (**L)-methionine** will exist as a zwitterion at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process(5). According to a classification scheme(6), an estimated BCF of 3(SRC), from its log Kow(2) and a regression-derived equation(7), suggests the potential for bioconcentration in aquatic organisms is low(SRC). Using a laboratory activated sludge system, (**L)-methionine** exhibited an 80% theoretical BOD reduction in 16 days, producing 3-mercaptopropionate, methanethiol and dimethylsulfide(8); this suggests that biodegradation may be an important environmental fate process in water(SRC).
[(1) Swann RL et al; Res Rev 85: 17-28 (1983) (2) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 15 (1995) (3) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm as of Feb 19, 2010. (4) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007. (5) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds, Boca Raton, FL: Lewis Publ (2000) (6) Franke C et al; Chemosphere 29: 1501-14 (1994) (7) Meylan WM et al; Environ Toxicol Chem 18: 664-72 (1999) (8) Engelbrecht RS, McKinney RE; Sew Indust Wastes 29: 1350-62 (1957)] **PEER REVIEWED**

Environmental Fate :
AQUATIC FATE: (**L)-Methionine** has been shown to degrade in sunlit natural water through a photo-sensitized oxidation involving singlet oxygen(1,2); assuming that the top meter of sunlit natural water has a singlet oxygen concn of 4X10-14 M, the photooxidation half-life for the reaction (**L)-methionine** with singlet oxygen has been estimated to be about 200 hr at pH 6-11(1); the near-surface photooxidation rate (via singlet oxygen) of (**L)-methionine** in Okefenokee Swamp water from Georgia is predicted to be about 3 hr(2). Bioconcentration and volatilization are not expected to important fate processes because of its high water solubility(SRC).
[(1) Haag WR, Holgne J; Environ Sci Technol 20: 341-8 (1986) (2) Zepp RG et al; Nature 267: 421-3 (1977)] **PEER REVIEWED**

Environmental Fate :
ATMOSPHERIC FATE: According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere(1), (**L)-methionine,** which has an estimated vapor pressure of 8.1X10-8 mm Hg at 25 deg C(SRC), determined from a fragment constant method(2), will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase (**L)-methionine** is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals(SRC); the half-life for this reaction in air is estimated to be 7.5 hours(SRC), calculated from its rate constant of 5.1X10-11 cu cm/molecule-sec at 25 deg C(SRC) that was derived using a structure estimation method(3). (**L)-Methionine** does not contain chromophores that absorb at wavelengths >290 nm(4) and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC).
[(1) Bidleman TF; Environ Sci Technol 22: 361-367 (1988) (2) Lyman WJ; p. 31 in Environmental Exposure From Chemicals Vol I, Neely WB, Blau GE, eds, Boca Raton, FL: CRC Press (1985) (3) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (4) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 8-12 (1990)] **PEER REVIEWED**

Environmental Biodegradation :
AEROBIC: In a laboratory activated sludge system, (**L)-methionine** had an 80% theoretical BOD reduction after 16 days of incubation(1). In a Warburg respirometer study using activated sludge, (**L)-methionine (**at a concn of 500 mg/L) had a theoretical BOD of 2.6% over a 24-hr incubation period(2). In an activated sludge system that had been acclimated to phenol, (**L)-methionine** had a theoretical oxidation of 16% after 12 hrs of aeration(3).
[(1) Engelbrecht RS, McKinney RE; Sew Indust Wastes 29: 1350-62 (1957) (2) Malaney GW, Gerhold RM; J Water Pollut Control Fed 41: R18-R33 (1969) (3) McKinney RE et al; Sew Indust Wastes 28: 547-57 (1956)] **PEER REVIEWED**

Environmental Abiotic Degradation :
The rate constant for the vapor-phase reaction of (**L)-methionine** with photochemically-produced hydroxyl radicals has been estimated as 6.1X10-11 cu cm/molecule-sec at 25 deg C(SRC) using a structure estimation method(1). This corresponds to an atmospheric half-life of about 7.5 hours at an atmospheric concentration of 5X10+5 hydroxyl radicals per cu cm(1). (**L)-Methionine** is not expected to undergo hydrolysis in the environment due to the lack of functional groups that hydrolyze under environmental conditions(3). (**L)-Methionine** does not contain chromophores that absorb at wavelengths >290 nm(3) and therefore is not expected to be susceptible to direct photolysis by sunlight(SRC).
[(1) Meylan WM, Howard PH; Chemosphere 26: 2293-99 (1993) (3) Lyman WJ et al; Handbook of Chemical Property Estimation Methods. Washington, DC: Amer Chem Soc pp. 7-4, 7-5, 8-12 (1990)] **PEER REVIEWED**

General Manufacturing Information :
... Available as d,**l-methionine** and as calcium salt of hydroxy analog of methionine. These products are used ... in animal feeds ... Relative biological value of these compared to **l-**

methionine ... evidence suggests equivalency on mole basis both in rats and man.
[Furia, T.E. (ed.). CRC Handbook of Food Additives. 2nd ed. Cleveland: The Chemical Rubber Co., 1972., p. 109] **PEER REVIEWED**

Volatilization from Water/Soil :
The pKa values of 2.28 and 9.21(1) indicate (**L)-methionine** will exist as a zwitterion at pH values of 5 to 9 and therefore volatilization from water surfaces is not expected to be an important fate process(2). (**L)-Methionine** is not expected to volatilize from dry soil surfaces(SRC) based upon an estimated vapor pressure of 8.1X10-8 mm Hg(SRC), determined from a fragment constant method(3).
[(1) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007. (2) Doucette WJ; pp. 141-188 in Handbook of Property Estimation Methods for Chemicals. Boethling RS, Mackay D, eds. Boca Raton, FL: Lewis Publ (2000) (3) Lyman WJ; p. 31 in Environmental Exposure From Chemicals Vol I, Neely WB, Blau GE, eds, Boca Raton, FL: CRC Press (1985)] **PEER REVIEWED**

Milk Concentrations :
ENVIRONMENTAL: The average (**L)-methionine** content of cows milk is reported as 86 mg/100 g(1). The average **(L)-methionine** content of human milk is reported as 19 mg/100 g(1).
[(1) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007.] **PEER REVIEWED**

Formulations/Preparations :
Racemate mixture of D- and **L- methionine**
[Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA2: 71 (1985)] **PEER REVIEWED**

U. S. Production :
World market for **L-methionine** in 1982: 150 tons; World market for DL-methionine in 1982: 110,000 tons
[Gerhartz, W. (exec ed.). Ullmann's Encyclopedia of Industrial Chemistry. 5th ed.Vol A1: Deerfield Beach, FL: VCH Publishers, 1985 to Present., p. VA2: 90 (1985)] **PEER REVIEWED**

Human Toxicity Excerpts :
/HUMAN EXPOSURE STUDIES/ ... Homocysteine levels in patients with inflammatory bowel disease (IBD) /were studied/ during fasting and after methionine load to determine the true prevalence of hyperhomocysteinemia and its relation with thrombotic events. METHODS: Prospective analysis of homocysteine levels in consecutive patients with IBD during fasting and 6-8 hours after an oral methionine load. Levels of folate and vitamin B12 were also determined. History of thrombotic events were recorded. RESULTS: Eighty-two patients with IBD ... were

included. Eighteen patients (22%) had hyperhomocysteinemia during fasting. Mean levels of homocysteine after methionine load were 20.4 +/- 18.1 umol/L (range, 1-79.7 umol/L), and 43 patients (52%) had hyperhomocysteinemia (> or =20 umol/**L) after methionine** load. Six patients (7.3%) had history of thrombosis. The homocysteine levels during fasting and after methionine load were significantly higher in patients with thrombotic events than in patients without thrombosis (15.5 +/- 3.7 umol/L vs. 6.6 +/- 6.5 umol/L; P = 0.002; 44.5 +/- 20.9 umol/L vs. 18.4 +/- 16.5 umol/L; P < 0.001, respectively)...
[Zepeda-Gomez S et al; Inflammatory Bowel Dis 14 (3): 383-8 (2008). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=17924554 **PEER REVIEWED**

Human Toxicity Excerpts :
/SIGNS AND SYMPTOMS/ Methionine supplements (5 g/day) for periods of weeks were reportedly innocuous in humans. ... Single oral doses of 7 g produced lethargy in six individuals and oral administration of 10.5 g of **L-methionine** to one produced nausea and vomiting. After an oral administration of 8 g/day of methionine (isomer not specified) for 4 days, serum folate concentrations were decreased in five otherwise healthy adults.
[NAS, Food and Nutrition Board, Institute of Medicine; Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press, Washington, D.C., pg. 726, 2009. Available from, as of March 10, 2010: http://www.nap.edu/catalog/10490.html **PEER REVIEWED**

Non-Human Toxicity Excerpts :
/LABORATORY ANIMALS: Acute Exposure/ A single dietary dose (2.7% of the diet) of **L-methionine** decreased body growth and also reduced food intake in rats.
[NAS, Food and Nutrition Board, Institute of Medicine; Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press, Washington, D.C., pg. 725, 2009. Available from, as of March 10, 2010: http://www.nap.edu/catalog/10490.html **PEER REVIEWED**

Non-Human Toxicity Excerpts :
/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Dietary excesses of **L-methionine (**2.7% of the diet) for 6, 13, or 20 days have been associated with erythrocyte engorgement and accumulation of hemosiderine in rats, and there was a depression of growth and splenic damage.
[NAS, Food and Nutrition Board, Institute of Medicine; Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press, Washington, D.C., pg. 725, 2009. Available from, as of March 10, 2010: http://www.nap.edu/catalog/10490.html **PEER REVIEWED**

Non-Human Toxicity Excerpts :
/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ Dietary intakes of 2 to 4% of

L-methionine caused slight changes in liver cells in rats and slight decreases in liver iron content. Darkened spleens caused by increases in iron deposition have been observed in weanling rats fed 1.8% methionine diets for 28 days.
[NAS, Food and Nutrition Board, Institute of Medicine; Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). National Academy Press, Washington, D.C., pg. 725, 2009. Available from, as of March 10, 2010: http://www.nap.edu/catalog/10490.html **PEER REVIEWED**

Non-Human Toxicity Excerpts :
/LABORATORY ANIMALS: Subchronic or Prechronic Exposure/ ... Male Wistar rats were fed either a **L-methionine**-supplemented (2.5 g/100 g) diet without changing any other dietary components or a control (0.86 g/100 g) diet for 7 weeks. It was found that methionine supplementation increased mitochondrial reactive oxygen species (ROS) generation and percent free radical leak in rat liver mitochondria but not in rat heart. In agreement with these data oxidative damage to mitochondrial DNA increased only in rat liver, but no changes were observed in five different markers of protein oxidation in both organs. The content of mitochondrial respiratory chain complexes and AIF (apoptosis inducing factor) did not change after the dietary supplementation while fatty acid unsaturation decreased. Methionine, S-AdenosylMethionine and S-AdenosylHomocysteine concentration increased in both organs in the supplemented group. These results show that methionine supplementation in the diet specifically increases mitochondrial ROS production and mitochondrial DNA oxidative damage in rat liver mitochondria offering a plausible mechanism for its hepatotoxicity.
[Gomez J et al; J Bioenergetics Biomembranes 41 (3): 309-21 (2009). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=19633937 **PEER REVIEWED**

Absorption, Distribution & Excretion :
... Rats were fed diets containing [(14)C-methyl]**l-methionine ...** with 6% of sodium formate, and conversion of (14)C into [(14)C]formate was measured in urine and exhaled air (as (14)CO2) ... Total oxidation of [(14)C-methyl] into CO2, amounted to 60-87% for methionine ...
[The Chemical Society. Foreign Compound Metabolism in Mammals. Volume 5: A Review of the Literature Published during 1976 and 1977. London: The Chemical Society, 1979., p. 435] **PEER REVIEWED**

Absorption, Distribution & Excretion :
Using a double-lumen tube perfusion system the rates of absorption of **L-methionine** and glucose from a 30 cm segment of the jejunum were estimated in eight relatively normal Zambian African subjects. The effect of each substrate on the absorption of the other has also been investigated. The solutions perfused were given at 12.0 ml/L/min, and contained 100 m-mole/L of **L-methionine,** 100 m-mole/L of **L-methionine** and 150 m-mole/L of glucose or 150 m-mole/L of glucose. The presence of glucose in the perfusing fluid did not significantly alter the mean absorption rate of methionine ... . Five subjects had a transitory psychiatric disturbance after the investigation. The cause of this is not clear, but was probably caused by the absorption of a break-down product of methionine from the large intestine.

[Cook GC; J Physiol 221 (3): 707-14 (1972). Available from, as of March 17, 2010:] **PEER REVIEWED** PubMed Abstract

Metabolism/Metabolites :
... Methionine ... is catabolized to a large extent independently of initial activation to S-adenosyl-**l-methionine.** The system for catabolism ... appears analogous to one that catalyses oxidation of S-methyl-l-cysteine methyl group ... The methyl group of methionine ... /has been/ shown ... to yield formate in vitro and in vivo.
[The Chemical Society. Foreign Compound Metabolism in Mammals. Volume 5: A Review of the Literature Published during 1976 and 1977. London: The Chemical Society, 1979., p. 435] **PEER REVIEWED**

Interactions :
Poor folate status is associated with cognitive decline and dementia in older adults. ... To better define the role of folate deficiency in cognitive dysfunction, ... rats /were fed/ folate-deficient diets (0 mg FA/kg diet) with or without supplemental **L-methionine** for 10 wk, followed by cognitive testing and tissue collection for hematological and biochemical analysis. Folate deficiency with normal methionine impaired spatial memory and learning; however, this impairment was prevented when the folate-deficient diet was supplemented with methionine...
[Troen AM et al; J Nutrition 138 (12): 2502-9 (2008). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=19022979 **PEER REVIEWED**

Interactions :
Rats were fed diets supplemented with 1% **L-methionine** with and without 2.5% various amino acids for 7 d to determine what amino acids other than glycine, serine, and cystine can suppress methionine-induced hyperhomocysteinemia. L-Glutamic acid, L-histidine, and L-arginine significantly suppressed methionine-induced enhancement of plasma homocysteine concentrations, but the mechanisms underlying the effect of these amino acids are thought not to be identical.
[Fukada S-I et al; Biosci Biotech Biochem 72 (7): 1940-3 (2008). Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=18603771 **PEER REVIEWED**

Interactions :
... Rats fed on a vitamin B12 (B12)-deficient diet containing 180 g soybean protein per kg diet showed marked histologic damage in their testes. /This paper reports/ the effect of B12-deficiency on B12-dependent methionine synthase in the rats' testes and the effect of methionine supplementation of the diet on testicular damage. Rats ... fed the soybean protein-based B12-deficient diet for 120 d ... were in serious B12-deficiency /as measured by/ urinary methylmalonic acid excretion and B12 content in tissues. Methionine synthase activity in the testis of the B12-deficient rats was less than 2% of that in B12-supplemented (control) rats. To complement disrupted methionine biosynthesis, methionine was supplied in the diet. A

supplement of 5 g D,**L-methionine** per kg diet to the B12-deficient diet did not affect urinary methylmalonic acid excretion of B12-deficient rats. The testicular histology of rats fed the methionine-supplemented B12-deficient diet was almost indistinguishable from that of control rats...
[Yamada K et al; J Nutrit Sci Vitaminology 53 (2): 95-101. Available from, as of March 17, 2010: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=17615995 **PEER REVIEWED**

Artificial Pollution Sources :
(**L)-Methionine's** production and use as nutritional supplement in animal feeds(1) may result in its release to the environment through various waste streams(SRC).
[(1) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). New York, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007.] **PEER REVIEWED**

Environmental Bioconcentration :
An estimated BCF of 3 was calculated in fish for (**L)-methionine(**SRC), using a log Kow of -1.87(1) and a regression-derived equation(2). According to a classification scheme(3), this BCF suggests the potential for bioconcentration in aquatic organisms is low(SRC).
[(1) Hansch C et al; Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Prof Ref Book. Heller SR, consult. ed., Washington, DC: Amer Chem Soc p. 125 (1995) (2) US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm as of Feb 19, 2010. (3) Franke C et al; Chemosphere 29: 1501-14 (1994)] **PEER REVIEWED**

Sediment/Soil Concentrations :
SOIL: (**L)-Methionine** concentrations in three soil horizons of an active landfill, the Conica Montemarte outside of Seville, Spain, were reported as 0.5 nmol/g (1.0-1.5 meters), 19.9 nmol/g (3.0-3.5 meters), and 38.2 nmol/g (6.5-7.0 meters), 6, 18, and 24 months after deposition respectively(1).
[(1) Gonzalez-Vila, FJ et al; Chemosphere 31: 2817-25 (1995)] **PEER REVIEWED**

Sediment/Soil Concentrations :
SEDIMENT: (**L)-Methionine** concentrations of 0.46 to 0.055 umol/g were detected in carbonate sediment samples collected from a lagoon of Fanning Island, an atoll in the Line Islands of the Central Pacific Ocean(1).
[(1) Muller PJ, Suess E; Geochim Casmochim Acta 41: 941-9 (1977)] **PEER REVIEWED**

Animal Concentrations :
Zebra mussels and clams sampled in November 1991 from Oneida Lake in NY State were found to have (**L)-methionine** levels of 1.66 and 1.58 g/100 g dry wt, respectively(1).

[(1) Secor CL et al; Chemosphere 26: 1559-75 (1993)] **PEER REVIEWED**

Body Burden :

The average (**L)-methionine** content of human milk is reported as 19 mg/100 g(1).
[(1) Drauz K et al; Ullmann's Encyclopedia of Industrial Chemistry. 7th ed. (2008). NY, NY: John Wiley & Sons; Amino Acids. Online Posting Date: Apr 15, 2007.] **PEER REVIEWED**

FDA Requirements :

L-Methionine is a food additive permitted for direct addition to food for human consumption, as long as 1) the quantity of the substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritive, or other technical effect in food, and 2) any substance intended for use in or on food is of appropriate food grade and is prepared and handled as a food ingredient.
[21 CFR 172.320 (USFDA); U.S. National Archives and Records Administration's Electronic Code of Federal Regulations. Available from, as of March 19, 2010: http://www.gpoaccess.gov/ecfr **PEER REVIEWED**

Neal, Arthur

From: dirk.hoehler@degussa.com%inter2 [dirk.hoehler@degussa.com] on behalf of dirk.hoehler@degussa.com
Sent: Monday, August 08, 2005 4:19 PM
To: National List
Subject: TM-05-02 - Methionine in Organic Poultry Production

Attachments: Life Cycle Analysis of DL-Methionine.pdf

Life Cycle Analysis of DL-Meth...

Arthur Neal
Director of Program Administration
National Organic program
USDA-AMS-TMP-NOP

Dear Mr. Neal,

we appreciate the extend of methionine use in organic poultry production until October 21, 2008.

Methionine is an essential amino acid. At present, there are no alternative organic methionine-rich ingredients available in sufficient quantities.

I'm attaching a paper entitled "Life cycle analysis of DL-methionine in broiler meat production". In this work, we had an independent institute evaluate the production methods and the effects of DL-methionine in poultry feeds. The results are very clear-cut, please have a look.

With best regards,

Dirk Hoehler

(See attached file: Life Cycle Analysis of DL-Methionine.pdf)

degussa.
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Special Issue
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Life cycle analysis of DL-methionine in broiler meat production

by
Dr. Michael Binder

Poultry / Environment

Life cycle analysis of DL-methionine in broiler meat production

Key information

- This paper presents a comparative assessment of the ecological impact of methionine supplementation in poultry meat production, using either synthetic DL-methionine or natural methionine derived from vegetable proteins.
- The study provides an energetic assessment of the entire life cycle of DL-methionine by process chain analysis.
- The comparison looks at the provision of 1 kg methionine for supplementation of a methionine-deficient broiler ration with DL-methionine vs. additional protein from methionine-rich oilseeds. The impact of alternative feeding strategies on the environment is analysed by measuring critical environmental parameters.
- Supplementation of 1 kg synthetic DL-methionine requires less than one sixth of the energy needed to provide the equivalent amount of methionine from

soybean meal or rapeseed meal. The significant reduction in energy use also leads to considerably lower process-relevant emissions, resulting in a sustained contribution towards reducing environmental pollution (eutrophication and acidification).

Life cycle analysis

The Feed Additives Business of Degussa AG is the world's largest producer of amino acids for animal nutrition. The main focus is on poultry and pig meat production, because amino acids are building blocks of dietary proteins and as such constitute a major factor for animal growth.

Degussa AG is quoted in the Dow Jones Sustainability Index, therefore the Feed Additives Division is committed to a proactive and consistent policy in the area of environmental and health protection. This is demonstrated by efforts to assess the environmental pros and cons of its products by means of life cycle analysis (ecobalance). DL-methionine was the first product to be subjected to ecobalance testing in collaboration with the "ifeu" (Institute for Energy and Environmental Research, Heidelberg). The ISO 14040 to 14042 international standards were the basis for these tests.

DL-methionine is manufactured exclusively from petrochemical raw materials by a chemical process. It is also the most significant amino acid in animal nutrition measured by annual production value. The amino acid is currently produced at three locations in three countries. Degussa's production facility at Wesseling/Germany also produces all essential intermediates on site.

Methionine is an essential amino acid, which plays a critical role in broiler production as the first-limiting amino acid. A methionine deficiency limits protein biosynthesis, which means that other amino acids supplied with the diet can no longer be utilised. Methionine deficiency can be corrected either by the provision of synthetic amino acids or by increasing the proportion of methionine-rich natural feed ingredients, such as soybean meal or rapeseed meal.

Supplementing livestock diets with synthetic methionine enables to supply this limiting amino acid with a high degree of precision. At the same time, extracted soybean meal or rapeseed meal, which are extensively used in poultry production as sources of protein (and methionine), can be replaced by grains. The provision of the two alternative methionine sources was compared over their entire life cycles.

Ecobalance testing allows an objective assessment of the ecological benefits of fine-tuning the methionine content of typical broiler rations by the addition of synthetic DL-methionine.

Equivalent feed mixtures as functional units

In the ecobalance evaluation of DL-methionine, alternative options for increasing the methionine content of complete broiler rations were compared. Firstly, a practical DL-methionine supplemented diet was formulated which covers the bird's nutrient requirements (Diet A). In Diets B and C, dietary methionine content was raised by increasing the proportion of soybean meal or rapeseed meal, respectively.

The energy content of all three feeds was adjusted by varied amounts of vegetable oil supplementations. All three diets were formulated to contain the same level of true digestible methionine. The system "Diet A" is based on the technical production of methionine as a speciality chemistry product. In the second and third option (B, C) the methionine content of the ration is adjusted by increasing the proportion of soybean meal or rapeseed meal. System B has to include the cultivation of soybeans in North and South America, transport, and processing in an oil mill. In order to provide a nutritionally equivalent mixture, this automatically entails a reduction of the cereal content – in this case wheat – in the feed mixture, thereby lowering the energy content of the ration. The reduced amount of wheat is credited to the system. In order to cover the requirement for metabolisable energy, the ration is supplemented with energy-rich soy oil. The manufacture of oil is also included in the balance calculation. Provision of the equivalent amount of methionine through rapeseed meal (the most important methionine-rich European protein source) is evaluated and balanced in the same way.

Additionally, each product system considers the specific emissions associated with the altered feed composition (higher protein content), i.e., emissions caused by poultry droppings and manure applied to the land.

Table 1: Broiler rations formulated based on meeting the methionine requirement by supplementing DL-methionine (A) vs. increased amounts of soybean meal (B) and rapeseed meal (C)

Ingredients (%)	Diet A	Diet B	Diet C
Wheat	63.0	45.2	44.3
Soybean meal	**27.0**	**43.0**	**27.0**
Rapeseed meal	–	–	**15.0**
Soybean oil	5.0	6.8	5.0
Rapeseed oil	-	-	3.8
Minerals & vitamins	5.0	5.0	5.0
DL-methionine	**0.06**	–	–
Metabolizable energy (ME, MJ/kg)	12.7	12.7	12.7
Crude protein (%)	**20.4**	**25.7**	**23.2**
True digestible methionine (%)	0.31	0.31	0.31

The ecological comparison of the two product systems is limited to considering the respective differences relative to the basal mixture A. Both balanced product systems are shown in the simplified flow chart in Figure 1. By standardising the differences in the respective feed formulations in Table 1 to 1 kg methionine in each case, we can draw up the following comparative equation for further consideration:

1 kg DL-methionine

=

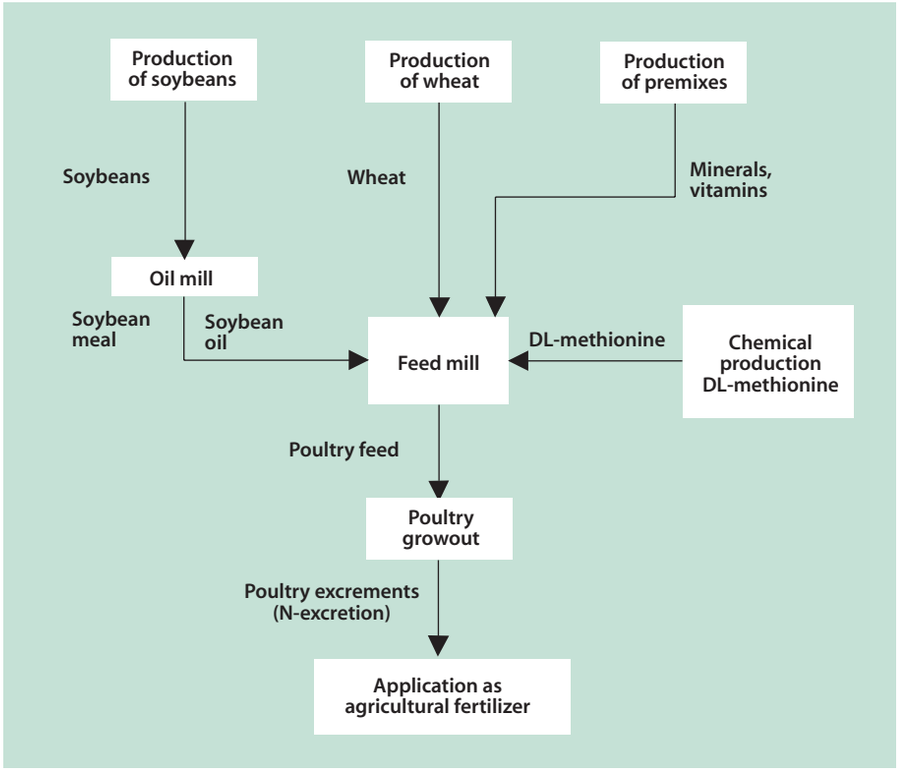
260 kg soybean meal
29 kg soybean oil
-288 kg wheat

=

250 kg rapeseed meal
29 kg rapeseed oil
-311 kg wheat

The manufacture of DL-methionine by the Degussa process (Figure 2) uses conventional petrochemical raw materials and comprises a series of synthesis steps in closed cycles with intensive recycling of by-products.

Figure 1: Life cycle of the various feed mixtures and the use of synthetic DL-methionine
(The material flows refer to a complete broiler diet with a 1 kg higher methionine content than the basal mixture; the supply of methionine from rapeseed meal can be assessed in the same way.)



Adverse environmental effects

Resource consumption / energy use

The consumption of natural resources is considered an impairment of the basis of human life. The conservation of resources plays an important role in all considerations about sustainable management. The term "resources" is usually confined to non-renewable mineral or fossil resources, which is closely related to the total consumption of energy. The "shortage" of a resource is traditionally adopted as a criterion for evaluating resource depletion. To determine whether a resource is in short supply, a relationship is established between the factors consumption, potential renewal and reserves for a specific geographical unit.

Figure 2: Simplified process chart of the chemical synthesis of DL-methionine from petrochemical raw materials by the Degussa process

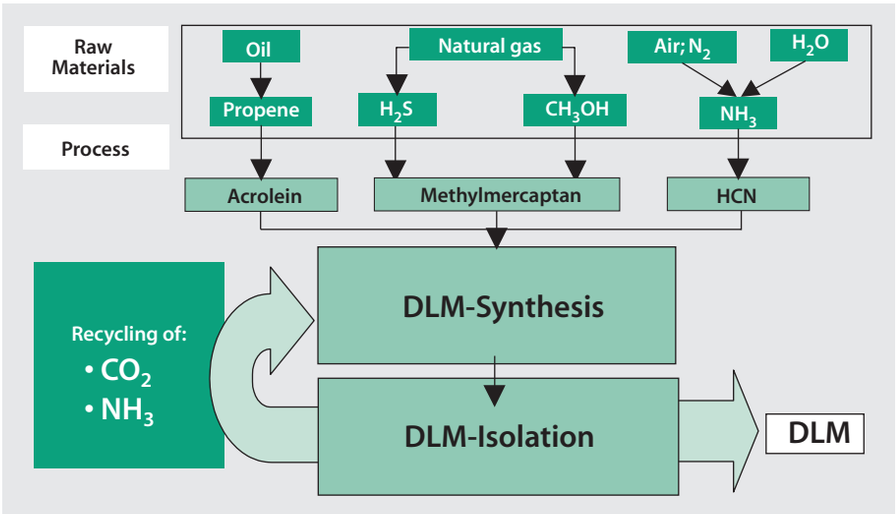
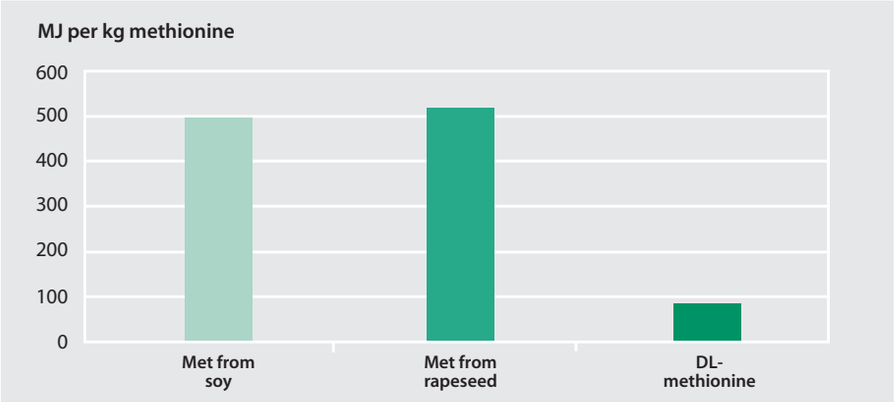


Figure 3: Cumulative energy consumption for 1 kg methionine. The methionine is derived either from chemical synthesis or from natural sources such as soybean meal and rapeseed meal (ifeu 2002).



Emissions

The different equivalent feed formulations in Table 1 show marked differences in their crude protein content while having identical energy and methionine contents. The nitrogen bound in the excess crude protein is not utilised efficiently by the animal's body but excreted and emitted into the environment as ammonia or nitrate. As a result, supplementation with synthetic methionine has distinct advantages over using higher proportions of plant-derived protein from soybean meal or rapeseed meal.

The chemical synthesis of 1 kg DL-methionine requires approximately 88 MJ of primary energy. Supplementation of the same amount of methionine via soybean meal or rapeseed meal involves a more than 6-fold greater energy consumption (Figure 3).

Besides distinctly lower emissions, chemical synthesis involves far less depletion of natural resources, expressed in crude oil equivalents per kg methionine. Due to differences in the way soybean meal and rapeseed meal are produced, the results for the two natural methionine sources differ, but are still well above the values for the chemical synthesis of DL-methionine (Figure 4). The high energy requirement for methionine from soy protein is due to the energy consumed during processing in the oil mill as well as due to that used for transporting the raw materials from the main producing countries of Brazil and USA to Europe (Figure 5). It should be noted that the energy consumption attributed to "Agriculture" reflects only the difference between 260 kg of soybean meal and 288 kg wheat. Therefore this becomes a very small figure.

Figure 4: Resource consumption in the provision of 1 kg methionine to supplement a methionine-deficient complete broiler ration. The methionine is derived either from chemical synthesis or from natural sources such as soybean meal and rapeseed meal (ifeu 2002).

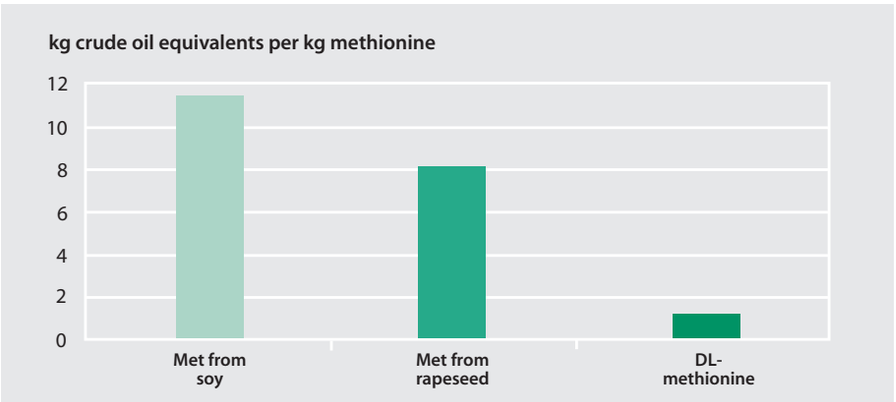
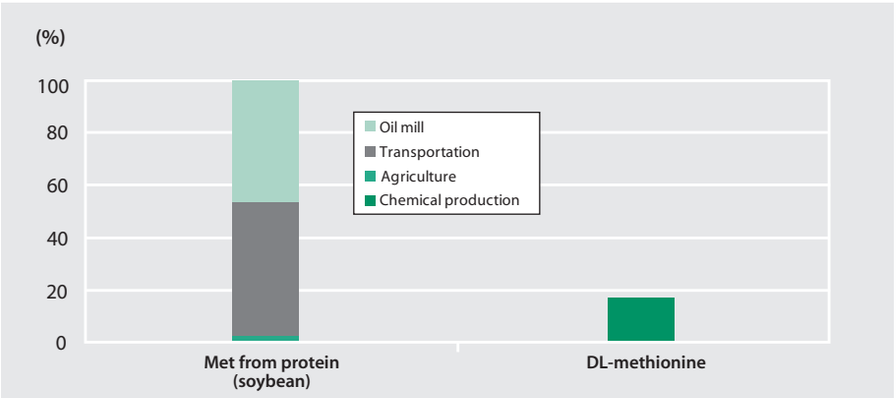


Figure 5: Relative specific contributions to energy consumption during different processing steps in the provision of 1 kg methionine (ifeu 2002)



Both the emission of ammonia (NH_3) into the air (terrestrial eutrophication; Figure 6) and the pollution of water with nitrates (NO_3^-, aquatic eutrophication; Figure 7) associated with the provision of 1 kg methionine can be greatly reduced.

Figure 6: Contributions to terrestrial eutrophication for the provision of 1 kg methionine either from chemical synthesis or from natural protein sources such as soybean meal or rapeseed meal (ifeu 2002)

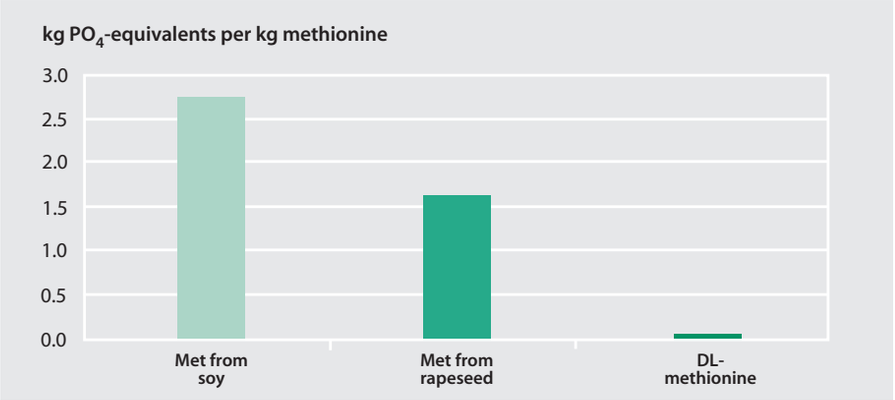
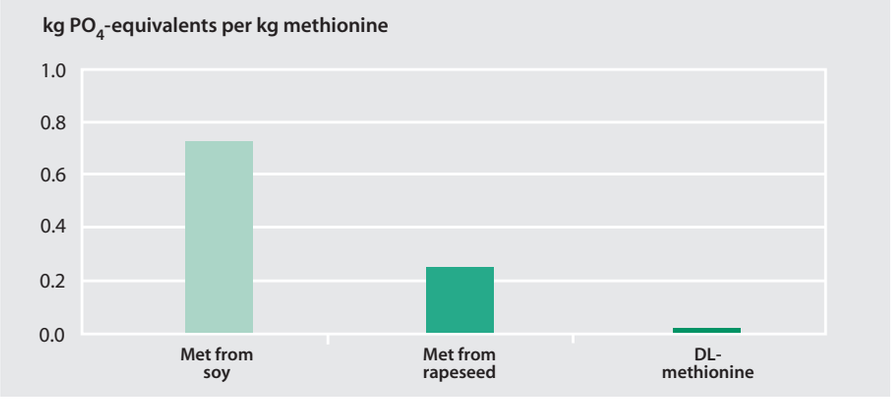


Figure 7: Contributions to aquatic eutrophication for the provision of 1 kg methionine either from chemical synthesis or from natural protein sources such as soybean meal or rapeseed meal (ifeu 2002)



Conclusion

Supplementation of broiler rations with synthetic DL-methionine represents a major contribution towards alleviating environmental pollution compared with the use of methionine-rich protein sources such as soybean meal or rapeseed meal. Firstly, the chemical production of methionine requires far less energy for achieving the same performance level with a given feed mixture. Secondly, it causes far lower environmental emissions. The lasting results are a saving of resources and a reduced emission of nitrogen and other compounds.

References

ifeu (2002): Ökobilanz für Methionin in der Geflügelmast / Life cycle analysis for methionine in broiler meat production. ifeu (Institute for Energy and Environmental Technology GmbH), Heidelberg.

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amino acids and more.

be safely used as a component of food, subject to the following restrictions:

(a) The additive is prepared with 50 percent Fischer-Tropsch process synthetic paraffin, meeting the definition and specifications of §172.615, and 50 percent of such synthetic paraffin to which is bonded succinic anhydride and succinic acid derivatives of isopropyl alcohol, polyethylene glycol, and polypropylene glycol. It consists of a mixture of the Fischer-Tropsch process paraffin (alkane), alkyl succinic anhydride, alkyl succinic anhydride isopropyl half ester, dialkyl succinic anhydride polyethylene glycol half ester, and dialkyl succinic anhydride polypropylene glycol half ester, where the alkane (alkyl) has a chain length of 30–70 carbon atoms and the polyethylene and polypropylene glycols have molecular weights of 600 and 260, respectively.

(b) The additive meets the following specifications: Molecular weight, 880–930; melting point, 215°–217 °F; acid number, 43–47; and saponification number, 75–78.

(c) It is used or intended for use as a protective coating or component of protective coatings for fresh grapefruit, lemons, limes, muskmelons, oranges, sweetpotatoes, and tangerines.

(d) It is used in an amount not to exceed that required to produce the intended effect.

§ 172.280 Terpene resin.

The food additive terpene resin may be safely used in accordance with the following prescribed conditions:

(a) The food additive is the beta-pinene polymer obtained by polymerizing terpene hydrocarbons derived from wood. It has a softening point of 112 °C–118 °C, as determined by ASTM method E28–67 (Reapproved 1982), ''Standard Test Method for Softening Point By Ring-and-Ball Apparatus,'' which is incorporated by reference. Copies may be obtained from the American Society for Testing Materials, 100 Barr Harbor Dr., West Conshohocken, Philadelphia, PA 19428-2959, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*.

(b) It is used or intended for use as follows:

(1) As a moisture barrier on soft gelatin capsules in an amount not to exceed 0.07 percent of the weight of the capsule.

(2) As a moisture barrier on powders of ascorbic acid or its salts in an amount not to exceed 7 percent of the weight of the powder.

[42 FR 14491, Mar. 15, 1977, as amended at 49 FR 10104, Mar. 19, 1984]

Subpart D—Special Dietary and Nutritional Additives

§ 172.310 Aluminum nicotinate.

Aluminum nicotinate may be safely used as a source of niacin in foods for special dietary use. A statement of the concentration of the additive, expressed as niacin, shall appear on the label of the food additive container or on that of any intermediate premix prepared therefrom.

§ 172.315 Nicotinamide-ascorbic acid complex.

Nicotinamide-ascorbic acid complex may be safely used in accordance with the following prescribed conditions:

(a) The additive is the product of the controlled reaction between ascorbic acid and nicotinamide, melting in the range 141 °C to 145 °C.

(b) It is used as a source of ascorbic acid and nicotinamide in multivitamin preparations.

§ 172.320 Amino acids.

The food additive amino acids may be safely used as nutrients added to foods in accordance with the following conditions:

(a) The food additive consists of one or more of the following individual amino acids in the free, hydrated or anhydrous form or as the hydrochloride, sodium or potassium salts:

L-Alanine
L-Arginine
L-Asparagine
L-Aspartic acid
L-Cysteine
L-Cystine
L-Glutamic acid

L-Glutamine
Aminoacetic acid (glycine)
L-Histidine
L-Isoleucine
L-Leucine
L-Lysine
DL-Methionine (not for infant foods)
L-Methionine
L-Phenylalanine
L-Proline
L-Serine
L-Threonine
L-Tryptophan
L-Tyrosine
L-Valine

(b) The food additive meets the following specifications:

(1) As found in ''Food Chemicals Codex,'' National Academy of Sciences/National Research Council (NAS/NRC), 3d Ed. (1981), which is incorporated by reference (Copies may be obtained from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal__register/code__of__federal__regulations/ibr__locations.html*.) for the following:

L-Alanine
L-Arginine
L-Arginine Monohydrochloride
L-Cysteine Monohydrochloride
L-Cystine
Aminoacetic acid (glycine)
L-Leucine
DL-Methionine
L-Methionine
L-Tryptophan
L-Phenylalanine
L-Proline
L-Serine
L-Threonine
Glutamic Acid Hydrochloride
L-Isoleucine
L-Lysine Monohydrochloride
Monopotassium *L*-glutamate
L-Tyrosine
L-Valine

(2) As found in ''Specifications and Criteria for Biochemical Compounds,'' NAS/NRC Publication, 3rd Ed. (1972), which is incorporated by reference (Copies are available from the Center for Food Safety and Applied Nutrition (HFS–200), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal__register/code__of__federal__regulations/ibr__locations.html*.) for the following:

L-Asparagine
L-Aspartic acid
L-Glutamine
L-Histidine

(c) The additive(s) is used or intended for use to significantly improve the biological quality of the total protein in a food containing naturally occurring primarily-intact protein that is considered a significant dietary protein source, provided that:

(1) A reasonable daily adult intake of the finished food furnishes at least 6.5 grams of naturally occurring primarily intact protein (based upon 10 percent of the daily allowance for the ''reference'' adult male recommended by the National Academy of Sciences in ''Recommended Dietary Allowances,'' NAS Publication No. 1694, 7th Ed. (1968), which is incorporated by reference. Copies are available from the Center for Food Safety and Applied Nutrition (HFS–200), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal__register/code__of__federal__regulations/ibr__locations.html*.

(2) The additive(s) results in a protein efficiency ratio (PER) of protein in the finished ready-to-eat food equivalent to casein as determined by the method specified in paragraph (d) of this section.

(3) Each amino acid (or combination of the minimum number necessary to achieve a statistically significant increase) added results in a statistically significant increase in the PER as determined by the method described in paragraph (d) of this section. The minimum amount of the amino acid(s) to achieve the desired effect must be used and the increase in PER over the primarily-intact naturally occurring protein in the food must be substantiated

as a statistically significant difference with at least a probability (P) value of less than 0.05.

(4) The amount of the additive added for nutritive purposes plus the amount naturally present in free and combined (as protein) form does not exceed the following levels of amino acids expressed as percent by weight of the total protein of the finished food:

	Percent by weight of total protein (expressed as free amino acid)
L-Alanine	6.1
L-Arginine	6.6
L-Aspartic acid (including L-asparagine)	7.0
L-Cystine (including L-cysteine)	2.3
L-Glutamic acid (including L-glutamine)	12.4
Aminoacetic acid (glycine)	3.5
L-Histidine	2.4
L-Isoleucine	6.6
L-Leucine	8.8
L-Lysine	6.4
L- and DL-Methionine	3.1
L-Phenylalanine	5.8
L-Proline	4.2
L-Serine	8.4
L-Threonine	5.0
L-Tryptophan	1.6
L-Tyrosine	4.3
L-Valine	7.4

(d) Compliance with the limitations concerning PER under paragraph (c) of this section shall be determined by the method described in sections 43.212–43.216, ''Official Methods of Analysis of the Association of Official Analytical Chemists,'' 13th Ed. (1980), which is incorporated by reference. Copies may be obtained from the AOAC INTERNATIONAL, 481 North Frederick Ave., suite 500, Gaithersburg, MD 20877, or may be examined at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202–741–6030, or go to: *http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html*. Each manufacturer or person employing the additive(s) under the provisions of this section shall keep and maintain throughout the period of his use of the additive(s) and for a minimum of 3 years thereafter, records of the tests required by this paragraph and other records required to assure effectiveness and compliance with this regulation and shall make such records available upon request at all reasonable hours by any officer or employee of the Food and Drug Administration, or any other officer or employee acting on behalf of the Secretary of Health and Human Services and shall permit such officer or employee to conduct such inventories of raw and finished materials on hand as he deems necessary and otherwise to check the correctness of such records.

(e) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the Act, the following:

(1) The name of the amino acid(s) contained therein including the specific optical and chemical form.

(2) The amounts of each amino acid contained in any mixture.

(3) Adequate directions for use to provide a finished food meeting the limitations prescribed by paragraph (c) of this section.

(f) The food additive amino acids added as nutrients to special dietary foods that are intended for use solely under medical supervision to meet nutritional requirements in specific medical conditions and comply with the requirements of part 105 of this chapter are exempt from the limitations in paragraphs (c) and (d) of this section and may be used in such foods at levels not to exceed good manufacturing practices.

[42 FR 14491, Mar. 15, 1977; 42 FR 56728, Oct. 28, 1977, as amended at 47 FR 11836, Mar. 19, 1982; 49 FR 10104, Mar. 19, 1984; 54 FR 24897, June 12, 1989; 59 FR 14550, Mar. 29, 1994; 61 FR 14480, Apr. 2, 1996]

§ 172.325 Bakers yeast protein.

Bakers yeast protein may be safely used in food in accordance with the following conditions:

(a) Bakers yeast protein is the insoluble proteinaceous material remaining after the mechanical rupture of yeast cells of *Saccharomyces cerevisiae* and removal of whole cell walls by centrifugation and separation of soluble cellular materials.

(b) The additive meets the following specifications on a dry weight basis:

(1) Zinc salts less than 500 parts per million (ppm) as zinc.

(2) Nucleic acid less than 2 percent.

may exist and so notifies the manufacturer, withdrawal of a product's exempt status shall be effective on the date of receipt of notification from the Director of the Center for Food Safety and Applied Nutrition. Additional or modified requirements, or the withdrawal of an exemption, apply only to those formulas that are manufactured after the compliance date. A postponement of the compliance date may be granted for good cause.

(3) FDA may decide that withdrawal of an exemption is necessary when, on the basis of its review under paragraph (d)(1) of this section, it concludes that quality control procedures are not adequate to ensure that the formula contains all required nutrients, that deviations in nutrient levels are not supported by generally accepted scientific, nutritional, or medical rationale, or that deviations from subpart B of this part are not necessary to provide appropriate directions for preparation and use of the infant formula, or that additional labeling information is necessary.

(4) FDA will use the following criteria in determining whether deviations from the requirements of this subpart are necessary and will adequately protect the public health:

(i) A deviation from the nutrient requirements of section 412(g) of the act or of regulations promulgated under section 412(a)(2) of the act is necessary to provide an infant formula that is appropriate for the dietary management of a specific disease, disorder, or medical condition;

(ii) For exempt infant formulas subject to paragraph (b) of this section, a deviation from the quality control procedures requirements of part 106 is necessary because of unusal or difficult technological problems in manufacturing the infant formula; and

(iii) A deviation from the labeling requirements of subpart B of this part is necessary because label information, including pictograms and symbols required by those regulations, could lead to inappropriate use of the product.

(e) *Notification requirements.* (1) Information required by paragraphs (b) and (c) of this section shall be submitted to Center for Food Safety and Applied Nutrition (HFS–830), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740.

(2) The manufacturer shall promptly notify FDA when the manufacturer has knowledge (as defined in section 412(c)(2) of the act) that reasonably supports the conclusion that an exempt infant formula that has been processed by the manufacturer and that has left an establishment subject to the control of the manufacturer may not provide the nutrients required by paragraph (b) or (c) of this section, or when there is an exempt infant formula that may be otherwise adulterated or misbranded and if so adulterated or misbranded presents a risk of human health. This notification shall be made, by telephone, to the Director of the appropriate FDA district office specified in part 5, subpart M of this chapter. After normal business hours (8 a.m. to 4:30 p.m.), the FDA emergency number, 301–443–1240, shall be used. The manufacturer shall send a followup written confirmation to the Center for Food Safety and Applied Nutrition (HFS–605), Food and Drug Administration, 5100 Paint Branch Pkwy., College Park, MD 20740, and to the appropriate FDA district office specified in part 5, subpart M of this chapter.

[50 FR 48187, Nov. 22, 1985, as amended at 61 FR 14479, Apr. 2, 1996; 66 FR 17358, Mar. 30, 2001; 66 FR 56035, Nov. 6, 2001; 67 FR 9585, Mar. 4, 2002; 69 FR 17291, Apr. 2, 2004]

Subpart D—Nutrient Requirements

§ 107.100 Nutrient specifications.

(a) An infant formula shall contain the following nutrients at a level not less than the minimum level specified and not more than the maximum level specified for each 100 kilocalories of the infant formula in the form prepared for consumption as directed on the container:

Nutrients	Unit of measurement	Minimum level	Maximum level
Protein	Grams	1.8	4.5
Fat	do	3.3	6.0
	Percent calories	30	54
Linoleic acid	Milligrams	300	.....
	Percent calories	2.7	.....
Vitamins			
Vitamin A	International Units	250	750

Nutrients	Unit of measurement	Minimum level	Maximum level
Vitamin D	do	40	100
Vitamin E	do	0.7	.....
Vitamin K	Micrograms	4	.....
Thiamine (vitamin B_1)	do	40	.....
Riboflavin (vitamin B_2)	do	60	.....
Vitamin B_6	do	35	.....
Vitamin B_{12}	do	0.15	.....
Niacin [1]	do	250	.....
Folic acid (folacin)	do	4	.....
Pantothenic acid	do	300	.....
Biotin [2]	do	1.5	.....
Vitamin C (ascorbic acid)	Milligrams	8	.....
Choline [2]	do	7	.....
Inositol [2]	do	4	.....
Minerals			
Calcium	do	60	.....
Phosphorus	do	30	.....
Magnesium	do	6	.....
Iron	do	0.15	3.0
Zinc	do	0.5	.....
Manganese	Micrograms	5	.....
Copper	Micrograms	60	.....
Iodine	do	5	75
Sodium	Milligrams	20	60
Potassium	do	80	200
Chloride	do	55	150

[1] The generic term "niacin" includes niacin (nicotinic acid) and niacinamide (nicotinamide).
[2] Required only for non-milk-based infant formulas.

In addition to the specifications established in the table in this paragraph for vitamins and minerals, the following also apply:

(b) Vitamin E shall be present at a level of at least 0.7 International Unit of vitamin E per gram of linoleic acid.

(c) Any vitamin K added shall be in the form of phylloquinone.

(d) Vitamin B_6 shall be present at a level of at least 15 micrograms of vitamin B_6 for each gram of protein in excess of 1.8 grams of protein per 100 kilocalories of infant formula in the form prepared for consumption as directed on the container.

(e) The ratio of calcium to phosphorus in infant formula in the form prepared for consumption as directed on the container shall be no less than 1.1 and not more than 2.0.

(f) Protein shall be present in an amount not to exceed 4.5 grams per 100 kilocalories regardless of quality, and not less than 1.8 grams per 100 kilocalories of infant formula in the form prepared for consumption as directed on the container when its biological quality is equivalent to or better than that of casein. If the biological quality of the protein is less than that of casein, the minimum amount of protein shall be increased proportionately to compensate for its lower biological quality. For example, an infant formula containing protein with a biological quality of 75 percent of casein shall contain at least 2.4 grams of protein (1.8/0.75). No protein with a biological quality less than 70 percent of casein shall be used.

[50 FR 45108, Oct. 30, 1985]

Subpart E—Infant Formula Recalls

SOURCE: 54 FR 4008, Jan. 27, 1989, unless otherwise noted.

§ 107.200 Food and Drug Administration-required recall.

When the Food and Drug Administration determines that an adulterated or misbranded infant formula presents a risk to human health, a manufacturer shall immediately take all actions necessary to recall that formula, extending to and including the retail level, consistent with the requirements of this subpart.

§ 107.210 Firm-initiated product removals.

(a) If a manufacturer has determined to recall voluntarily from the market an infant formula that is not subject to § 107.200 but that otherwise violates the laws and regulations administered by the Food and Drug Administration (FDA) and that would be subject to legal action, the manufacturer, upon prompt notification to FDA, shall administer such voluntary recall consistent with the requirements of this subpart.

(b) If a manufacturer has determined to withdraw voluntarily from the market an infant formula that is adulterated or misbranded in only a minor way and that would not be subject to legal action, such removal from the market is deemed to be a market withdrawal, as defined in § 7.3(j) of this chapter. As required by § 107.240(a), the manufacturer shall promptly notify FDA of such violative formula and may, but is not required to, conduct such market withdrawal consistent with the requirements of this subpart pertaining to product recalls.

STANDARD FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS

CODEX STAN 72 – 1981

SECTION A: REVISED STANDARD FOR INFANT FORMULA

PREAMBLE

This standard is divided into two sections. Section A refers to Infant Formula, and Section B deals with Formulas for Special Medical Purposes Intended for Infants.

1. SCOPE

1.1 This section of the Standard applies to infant formula in liquid or powdered form intended for use, where necessary, as a substitute for human milk in meeting the normal nutritional requirements of infants.

1.2 This section of the Standard contains compositional, quality and safety requirements for Infant Formula.

1.3 Only products that comply with the criteria laid down in the provisions of this section of this Standard would be accepted for marketing as infant formula. No product other than infant formula may be marketed or otherwise represented as suitable for satisfying by itself the nutritional requirements of normal healthy infants during the first months of life.

1.4 The application of this section of the Standard should take into account the recommendations made in the International Code of Marketing of Breast-milk Substitutes (1981), the Global Strategy for Infant and Young Child Feeding and World Health Assembly resolution WHA54.2 (2001).

2. DESCRIPTION

2.1 Product Definition

2.1.1 Infant formula means a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.

2.1.2 The product is so processed by physical means only and so packaged as to prevent spoilage and contamination under all normal conditions of handling, storage and distribution in the country where the product is sold.

2.2 Other Definitions

The term *infant* means a person not more than 12 months of age.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Essential Composition

3.1.1 Infant formula is a product based on milk of cows or other animals or a mixture thereof and/or other ingredients which have been proven to be suitable for infant feeding. The nutritional safety and adequacy of infant formula shall be scientifically demonstrated to support growth and development of infants. All ingredients and food additives shall be gluten-free.

3.1.2 Infant formula prepared ready for consumption in accordance with instructions of the manufacturer shall contain per 100 ml not less than 60 kcal (250 kJ) and not more than 70 kcal (295 kJ) of energy.

3.1.3 Infant formula prepared ready for consumption shall contain per 100 kcal (100 kJ) the following nutrients with the following minimum and maximum or guidance upper levels (GUL)[1], as appropriate. The general principles for establishing these levels are identified in Annex II of this standard.

a) Protein[2), 3), 4)]

Unit	Minimum	Maximum	GUL
g/100 kcal	1.8 [5), 6)]	3.0	-
g/100 kJ	0.45 [5), 6)]	0.7	-

[2)] For the purpose of this standard, the calculation of the protein content of the final product prepared ready for consumption should be based on N x 6.25, unless a scientific justification is provided for the use of a different conversion factor for a particular product. The protein levels set in this standard are based on a nitrogen conversion factor of 6.25. The value of 6.38 is generally established as a specific factor appropriate for conversion of nitrogen to protein in other milk products, and the value of 5.71 as a specific factor for conversion of nitrogen to protein in other soy products.

[3)] For an equal energy value the formula must contain an available quantity of each essential and semi-essential amino acid at least equal to that contained in the reference protein (breast-milk as defined in Annex I); nevertheless for calculation purposes, the concentrations of tyrosine and phenylalanine may be added together. The concentrations of methionine and cysteine may be added together if the ratio is less than 2:1; in the case that the ratio is between 2:1 and 3:1 the suitability of the formula has to be demonstrated by clinical testing.

[4)] Isolated amino acids may be added to Infant Formula only to improve its nutritional value for infants. Essential and semi-essential amino acids may be added to improve protein quality, only in amounts necessary for that purpose. Only L-forms of amino acids shall be used.

[5)] The minimum value applies to cows' milk protein. For infant formula based on non-cows' milk protein other minimum values may need to be applied. For infant formula based on soy protein isolate, a minimum value of 2.25 g/100 kcal (0.5 g/100 kJ) applies.

[6)] Infant formula based on non-hydrolysed milk protein containing less than 2 g protein/ 100 kcal and infant formula based on hydrolysed protein containing less than 2.25 g protein/ 100 kcal should be clinically evaluated.

b) Lipids

Total fat [7, 8)]

Unit	Minimum	Maximum	GUL
g/100 kcal	4.4	6.0	-
g/100 kJ	1.05	1.4	-

[7)] Commercially hydrogenated oils and fats shall not be used in infant formula.

[8)] Lauric and myristic acids are constituents of fats, but combined shall not exceed 20% of total fatty acids. The content of trans fatty acids shall not exceed 3 % of total fatty acids. Trans fatty acids are endogenous components of milk fat. The acceptance of up to 3% of trans fatty acids is intended to allow for the use of milk fat in infant formulae. The erucic acid content shall not exceed 1% of total fatty acids. The total content of phospholipids should not exceed 300 mg/100 kcal (72 mg/100 kJ).

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[1] Guidance upper levels are for nutrients without sufficient information for a science-based risk assessment. These levels are values derived on the basis of meeting nutritional requirements of infants and an established history of apparent safe use. They may be adjusted based on relevant scientific or technological progress. The purpose of the GULs is to provide guidance to manufacturers and they should not be interpreted as goal values. Nutrient contents in infant formulas should usually not exceed the GULs unless higher nutrient levels cannot be avoided due to high or variable contents in constituents of infant formulas or due to technological reasons. When a product type or form has ordinarily contained lower levels than the GULs, manufacturers should not increase levels of nutrients to approach the GULs.

Linoleic acid

Unit	Minimum	Maximum	GUL
mg/100 kcal	300	-	1400
mg/100 kJ	70	-	330

α-Linolenic acid

Unit	Minimum	Maximum	GUL
mg/100 kcal	50	N.S.*	-
mg/100 kJ	12	N.S.	-

*N.S. = not specified

Ratio linoleic/ α-linolenic acid

Min	Max
5:1	15:1

c) Carbohydrates

Total carbohydrates[9)]

Unit	Minimum	Maximum	GUL
g/100 kcal	9.0	14.0	-
g/100 kJ	2.2	3.3	-

[9)] Lactose and glucose polymers should be the preferred carbohydrates in formula based on cows' milk protein and hydrolysed protein. Only precooked and/or gelatinised starches gluten-free by nature may be added to Infant Formula up to 30% of total carbohydrates and up to 2 g/100 ml.

Sucrose, unless needed, and the addition of fructose as an ingredient should be avoided in infant formula, because of potential life-threatening symptoms in young infants with unrecognised hereditary fructose intolerance.

d) Vitamins

Vitamin A

Unit	Minimum	Maximum	GUL
µg RE[10)]/100 kcal	60	180	-
µg RE[10)]/100 kJ	14	43	-

[10)] expressed as retinol equivalents (RE).

1 µg RE = 3.33 IU Vitamin A = 1 µg all-trans retinol. Retinol contents shall be provided by preformed retinol, while any contents of carotenoids should not be included in the calculation and declaration of vitamin A activity.

Vitamin D_3

Unit	Minimum	Maximum	GUL
µg[11)]/100 kcal	1	2.5	-
µg[11)]/100 kJ	0.25	0.6	-

[11)] Calciferol. 1 µg calciferol = 40 IU vitamin D

Vitamin E

Unit	Minimum	Maximum	GUL
mg α-TE[12)]/100 kcal	0.5[13)]	-	5
mg α-TE[12)]/100 kJ	0.12[13)]	-	1.2

[12)] 1 mg α-TE (alpha-tocopherol equivalent) = 1 mg d-α-tocopherol

[13)] Vitamin E content shall be at least 0.5 mg α-TE per g PUFA, using the following factors of equivalence to adapt the minimal vitamin E content to the number of fatty acid double bonds in the formula: 0.5 mg -TE/g linoleic acid (18:2 n-6); 0.75 α-TE/g α-linolenic acid (18:3 n-3); 1.0 mg α-TE/g arachidonic acid (20:4 n-6); 1.25 mg α-TE/g eicosapentaenoic acid (20:5 n-3); 1.5 mg α-TE/g docosahexaenoic acid (22:6 n-3).

Vitamin K

Unit	Minimum	Maximum	GUL
µg/100 kcal	4	-	27
µg/100 kJ	1	-	6.5

Thiamin

Unit	Minimum	Maximum	GUL
µg/100 kcal	60	-	300
µg/100 kJ	14	-	72

Riboflavin

Unit	Minimum	Maximum	GUL
µg/100 kcal	80	-	500
µg/100 kJ	19	-	119

Niacin[14)]

Unit	Minimum	Maximum	GUL
µg/100 kcal	300	-	1500
µg/100 kJ	70	-	360

[14)] Niacin refers to preformed niacin.

Vitamin B_6

Unit	Minimum	Maximum	GUL
µg/100 kcal	35	-	175
µg/100 kJ	8.5	-	45

Vitamin B_{12}

Unit	Minimum	Maximum	GUL
µg/100 kcal	0.1	-	1.5
µg/100 kJ	0.025	-	0.36

Pantothenic acid

Unit	Minimum	Maximum	GUL
µg/100 kcal	400	-	2000
µg/100 kJ	96	-	478

Folic acid

Unit	Minimum	Maximum	GUL
µg/100 kcal	10	-	50
µg/100 kJ	2.5	-	12

Vitamin C[15)]

Unit	Minimum	Maximum	GUL
mg/100 kcal	10	-	70[16)]
mg/100 kJ	2.5	-	17[16)]

[15)] expressed as ascorbic acid

[16)] This GUL has been set to account for possible high losses over shelf-life in liquid formulas; for powdered products lower upper levels should be aimed for.

Biotin

Unit	Minimum	Maximum	GUL
µg/100 kcal	1.5	-	10
µg/100 kJ	0.4	-	2.4

e) Minerals and Trace Elements

Iron

Unit	Minimum	Maximum	GUL[17)]
mg/100 kcal	0.45	-	-
mg/100 kJ	0.1	-	-

[17)]Levels may need to be determined by national authorities.

Calcium

Unit	Minimum	Maximum	GUL
mg/100 kcal	50	-	140
mg/100 kJ	12	-	35

Phosphorus

Unit	Minimum	Maximum	GUL
mg/100 kcal	25	-	100[18)]
mg/100 kJ	6	-	24[18)]

[18)] This GUL should accommodate higher needs with soy formula.

Ratio calcium/ phosphorus

Min	Max
1:1	2:1

Magnesium

Unit	Minimum	Maximum	GUL
mg/100 kcal	5	-	15
mg/100 kJ	1.2	-	3.6

Sodium

Unit	Minimum	Maximum	GUL
mg/100 kcal	20	60	-
mg/100 kJ	5	14	-

Chloride

Unit	Minimum	Maximum	GUL
mg/100 kcal	50	160	-
mg/100 kJ	12	38	-

Potassium

Unit	Minimum	Maximum	GUL
mg/100 kcal	60	180	-
mg/100 kJ	14	43	-

Manganese

Unit	Minimum	Maximum	GUL
µg/100 kcal	1	-	100
µg/100 kJ	0.25	-	24

Iodine

Unit	Minimum	Maximum	GUL
µg/100 kcal	10	-	60
µg/100 kJ	2.5	-	14

Selenium

Unit	Minimum	Maximum	GUL
µg/100 kcal	1	-	9
µg/100 kJ	0.24	-	2.2

Copper [19)]

Unit	Minimum	Maximum	GUL
µg/100 kcal	35	-	120
µg/100 kJ	8.5	-	29

[19)] Adjustment may be needed in these levels for infant formula made in regions with a high content of copper in the water supply.

Zinc

Unit	Minimum	Maximum	GUL
mg/100 kcal	0.5	-	1.5
mg/100 kJ	0.12	-	0.36

f) Other Substances

Choline

Unit	Minimum	Maximum	GUL
mg/100 kcal	7	-	50
mg/100 kJ	1.7	-	12

Myo-Inositol

Unit	Minimum	Maximum	GUL
mg/100 kcal	4	-	40
mg/100 kJ	1	-	9.5

L-Carnitine

Unit	Minimum	Maximum	GUL
mg/100 kcal	1.2	N.S.	-
mg/100 kJ	0.3	N.S.	-

3.2 Optional ingredients

3.2.1 In addition to the compositional requirements listed under 3.1.3, other ingredients may be added in order to provide substances ordinarily found in human milk and to ensure that the formulation is suitable as the sole source of nutrition for the infant or to provide other benefits that are similar to outcomes of populations of breastfed babies.

3.2.2 The suitability for the particular nutritional uses of infants and the safety of these substances shall be scientifically demonstrated. The formula shall contain sufficient amounts of these substances to achieve the intended effect, taking into account levels in human milk.

3.2.3 The following substances may be added in conformity with national legislation, in which case their content per 100 kcal (100 kJ) in the Infant Formula ready for consumption shall not exceed:

Taurine

Unit	Minimum	Maximum	GUL
mg/100 kcal	-	12	-
mg/100 kJ	-	3	-

Total nucleotides

Levels may need to be determined by national authorities.

Docosahexaenoic Acid [20)]

Unit	Minimum	Maximum	GUL
% of fatty acids	-	-	0.5

[20)] If docosahexaenoic acid (22:6 n-3) is added to infant formula, arachidonic acid (20:4 n-6) contents should reach at least the same concentration as DHA. The content of eicosapentaenoic acid (20:5 n-3), which can occur in sources of LC-PUFA, should not exceed the content of docosahexaenoic acid. National authorities may deviate from the above conditions, as appropriate for the nutritional needs.

3.2.4 Only L(+)lactic acid producing cultures may be used.

3.3 Fluoride

Fluoride should not be added to infant formula. In any case its level should not exceed 100 µg /100 kcal (24µg/100 kJ) in infant formula prepared ready for consumption as recommended by the manufacturer.

3.4 Vitamin Compounds and Mineral Salts

Vitamins and minerals added in accordance with Section 3.1.3 (d and e) and other nutrients added in accordance with 3.2.1 should be selected from the Advisory Lists of Mineral Salts and Vitamin Compounds for Use in Foods for Infants and Children (CAC/GL 10-1979).

3.5 Consistency and Particle Size

When prepared according to the label directions for use, the product shall be free of lumps and of large coarse particles and suitable for adequate feeding of young infants.

3.6 Purity Requirements

All ingredients shall be clean, of good quality, safe and suitable for ingestion by infants. They shall conform with their normal quality requirements, such as colour, flavour and odour.

3.7 Specific Prohibitions

The product and its component shall not have been treated by ionizing irradiation.

4. FOOD ADDITIVES

Only the food additives listed in this Section or in the Codex Advisory List of Mineral Salts and Vitamin Compounds for Use in Foods for Infants and Children (CAC/GL 10-1979) may be present in the foods described in section 2.1 of this Standard, as a result of carry-over from a raw material or other ingredient (including food additive) used to produce the food, subject to the following conditions:

a) The amount of the food additive in the raw materials or other ingredients (including food additives) does not exceed the maximum level specified; and

b) The food into which the food additive is carried over does not contain the food additive in greater quantity than would be introduced by the use of the raw materials or ingredients under good manufacturing practice, consistent with the provisions on carry-over in the Preamble of the General Standard for Food Additives (CAC/STAN 192-1995).

The following food additives are acceptable for use in the preparation of infant formula, as described in Section 2.1 of this Standard (in 100 ml of product, ready for consumption prepared following manufacturer's instructions, unless otherwise indicated):

INS	Additive	Maximum level in 100 ml of the product ready for consumption
4.1 Thickeners		
412	Guar gum	0.1 g in liquid formulas containing hydrolysed protein
410	Carob bean gum (Locust bean gum)	0.1 g in all types of infant formula
1412	Distarch phosphate	0.5 g singly or in combination in soy-based infant formula only 2.5 g singly or in combination in hydrolyzed protein- and/or amino acid based infant formula only
1414	Acetylated distarch phosphate	
1413	Phosphated distarch phosphate	
1440	Hydroxypropyl starch	
407	Carrageenan[2]	0.03 g in regular milk-and soy-based liquid infant formula only 0.1 g in hydrolysed protein- and/or amino acid based liquid infant formula only
4.2 Emulsifiers		
322	Lecithins	0.5 g in all types of infant formula[22)]
471	Mono- and diglycerides	0.4 g in all types of infant formula[22)]
4.3 Acidity Regulators		
524	Sodium hydroxide	0.2 g singly or in combination and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula
500ii	Sodium hydrogen carbonate	0.2 g singly or in combination and within the limits for sodium, potassium and calcium in section 3.1.3 (e) in all types of infant formula
500i	Sodium carbonate	
525	Potassium hydroxide	
501ii	Potassium hydrogen carbonate	
501i	Potassium carbonate	
526	Calcium hydroxide	
270	L(+) lactic acid	Limited by GMP in all types of infant formula

[2] Not endorsed by the 39th Session of the CCFA. JECFA evaluation is pending. national authorities may restrict its use until JECFA evaluation has been completed.

[22)] If more than one of the substances INS 322, 471 are added the maximum level for each of those substances is lowered with the relative part as present of the other substances

INS	Additive	Maximum level in 100 ml of the product ready for consumption
330	Citric acid	Limited by GMP in all types of infant formula
331i	Sodium dihydrogen citrate	Limited by GMP in all types of infant formula
331iii	Trisodium citrate	Limited by GMP in all types of infant formula
332	Potassium citrate	Limited by GMP in all types of infant formula
4.4 Antioxidants		
307b	Mixed tocopherol concentrate	1 mg in all types of infant formula singly or in combination
304i	Ascorbyl palmitate	1 mg in all types of infant formula singly or in combination
4.9 Packaging Gases		
290	Carbon dioxide	GMP
941	Nitrogen	

5. CONTAMINANTS

5.1 Pesticide Residues

The product shall be prepared with special care under good manufacturing practices, so that residues of those pesticides which may be required in the production, storage or processing of the raw materials or the finished food ingredient do not remain, or, if technically unavoidable, are reduced to the maximum extent possible.

5.2 Other Contaminants

The product shall not contain contaminants or undesirable substances (e.g. biologically active substances) in amounts which may represent a hazard to the health of the infant. The product covered by the provisions of the Standard shall comply with those maximum residue limits and maximum levels established by the Codex Alimentarius Commission.

	Maximum level
Lead	0.02 mg/kg (in the ready-to-use product)

6. HYGIENE

6.1 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice - General Principles of Food Hygiene (CAC/RCP 1-1969), and other relevant Codex texts such as the Recommended International Code of Hygienic Practice for Foods for Infants and Children (CAC/RCP 21-1979).

6.2 The products should comply with any microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997).

7. PACKAGING

7.1 The product shall be packed in containers which will safeguard the hygienic and other qualities of the food. When in liquid form, the product shall be packed in hermetically sealed containers; nitrogen and carbon dioxide may be used as packing media.

7.2 The containers, including packaging materials, shall be made only of substances which are safe and suitable for their intended uses. Where the Codex Alimentarius Commission has established a standard for any such substance used as packaging materials, that standard shall apply.

8. FILL OF CONTAINER

In the case of products in ready-to-eat form, the fill of container shall be:

(i) not less than 80% v/v for products weighing less than 150 g (5 oz.);

(ii) not less than 85% v/v for products in the weight range 150-250 g (5-8 oz.); and

(iii) not less than 90% v/v for products weighing more than 250 g (8 oz.) of the water capacity of the container. The water capacity of the container is the volume of distilled water at 20° C which the sealed container will hold completely filled.

9. LABELLING

The requirements of the Codex General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985), the Codex Guidelines on Nutrition Labelling (CAC/GL 2-1985) and the Guidelines for Use of Nutrition and Health Claims apply to infant formula and formula for special medical purposes for infants. These requirements include a prohibition on the use of nutrition and health claims for foods for infants and young children except where specifically provided for in relevant Codex Standards or national legislation. In addition to these requirements the following specific provisions apply:

9.1 The Name of the Food

9.1.1 The text of the label and all other information accompanying the product shall be written in the appropriate language(s).

9.1.2 The name of the product shall be either "Infant Formula" or any appropriate designation indicating the true nature of the product, in accordance with national usage.

9.1.3 The sources of protein in the product shall be clearly shown on the label.

9.1.4 If cows' milk is the only source of protein, the product may be labelled "Infant Formula Based on Cows' Milk".

9.1.5 A product which contains neither milk or any milk derivative shall be labelled "contains no milk or milk products" or an equivalent phrase.

9.2 List of Ingredients

9.2.1 A complete list of ingredients shall be declared on the label in descending order of proportion except that in the case of added vitamins and minerals, these ingredients may be arranged as separate groups for vitamins and minerals. Within these groups the vitamins and minerals need not be listed in descending order of proportion.

9.2.2 The specific name shall be declared for ingredients of animal or plant origin and for food additives. In addition, appropriate class names for these ingredients and additives may be included on the label.

9.3 Declaration of Nutritive Value

The declaration of nutrition information shall contain the following information which should be in the following order:

a) the amount of energy, expressed in kilocalories (kcal) and/or kilojoules (kJ), and the number of grammes of protein, carbohydrate and fat per 100 grammes or per 100 milliliters of the food as sold as well as per 100 milliliters of the food ready for use, when prepared according to the instructions on the label.

b) the total quantity of each vitamin, mineral, choline as listed in paragraph 3.1.3 and any other ingredient as listed in paragraph 3.2 of this Standard per 100 grammes or per 100 milliliters of the food as sold as well as per 100 milliliters of the food ready for use, when prepared according to the instructions on the label.

c) In addition, the declaration of nutrients in a) and b) per 100 kilocalories (or per 100 kilojoules) is permitted.

9.4 Date Marking and Storage Instructions

9.4.1 The date of minimum durability (preceded by the words "best before") shall be declared by the day, month and year in uncoded numerical sequence except that for products with a shelf-life of more than three months, the month and year will suffice. The month may be indicated by letters in those countries where such use will not confuse the consumer.

In the case of products requiring a declaration of month and year only, and the shelf-life of the product is valid to the end of a given year, the expression "end (stated year)" may be used as an alternative.

9.4.2 In addition to the date, any special conditions for the storage of the food shall be indicated if the validity of the date depends thereon.

Where practicable, storage instructions shall be in close proximity to the date marking.

9.5 Information for Use

9.5.1 Products in liquid form may be used either directly or in the case of concentrated liquid products, must be prepared with water that is safe or has been rendered safe by previous boiling before feeding, according to directions for use. Products in powder form should be reconstituted with water that is safe or has been rendered safe by previous boiling for preparation. Adequate directions for the appropriate preparation and handling should be in accordance with Good Hygienic Practice.

9.5.2 Adequate directions for the appropriate preparations and use of the product, including its storage and disposal after preparation, i.e. that formula remaining after feeding should be discarded, shall appear on the label and in any accompanying leaflet.

9.5.3 The label shall carry clear graphic instructions illustrating the method of preparation of the product.

9.5.4 The directions should be accompanied by a warning about the health hazards of inappropriate preparation, storage and use.

9.5.5 Adequate directions regarding the storage of the product after the container has been opened, shall appear on the label and in any accompanying leaflet.

9.6 Additional Labelling Requirements

9.6.1 Labels should not discourage breastfeeding. Each container label shall have a clear, conspicuous and easily readable message which includes the following points:

a) the words "important notice" or their equivalent;

b) the statement "Breast milk is the best food for your baby" or a similar statement as to the superiority of breastfeeding or breast milk;

c) a statement that the product should only be used on advice of a independent health worker as to the need for its use and the proper method of use.

9.6.2 The label shall have no pictures of infants and women nor any other picture or text which idealizes the use of infant formula.

9.6.3 The terms "humanized", "maternalized" or other similar terms shall not be used.

9.6.4 Information shall appear on the label to the effect that infants should receive complementary foods in addition to the formula, from an age that is appropriate for their specific growth and development needs, as advised by an independent health worker, and in any case from the age over six months.

9.6.5 The products shall be labelled in such a way as to avoid any risk of confusion between infant formula, follow-up formula, and formula for special medical purposes.

10. METHODS OF ANALYSIS AND SAMPLING[3]

[3] To be finalized.

Annex I

Essential and semi-essential amino acids in breast milk*

For the purpose of this Standard the essential and semi-essential amino acids in human milk from published studies which report measurements of the total nitrogen content and/or the calculation method of the protein content, expressed as mg per g of nitrogen and as mg per 100 kcal are listed.

The average level of an amino acid (mg per g of nitrogen) from each study was used to calculate the corresponding amino acid content per 100 kcal of an infant formula with the minimum protein content of 1.8 g/ 100 kcal accepted in this Standard (mg amino acid/g nitrogen in breast-milk divided by the nitrogen conversion factor of 6.25 and multiplied by 1.8).

The mean of the sums of the average amino acid levels from all studies was converted in the same manner to the average amounts of an amino acid per g of protein (total nitrogen x 6.25) and per 100 kcal of energy (columns 19 and 20 of the table).

National authorities may use all of the listed values.

** Adapted from Koletzko B, Baker S, Cleghorn G, et al, Global standard for the composition of infant formula: Recommendations of ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr. 2005;41:584-599.*

	Lönnerdal &Forsum (1985)		**Darragh & Moughan (1998)**		**Bindels & Harzer (1985)**		**Janas et al. (1987)**		**Villalpando et al. (1998)**				**Räihä et al. (2002) mod Nayman et al. (1979)**		**Yonekubo et al. (1991)**		**Mean of all amino acids contents**		
	Pooled banked milk at 4-16 weeks		**Pooled over 20 days at 10-14 weeks (n=20)**		**24 hours, pooled at 5 weeks (n=10)**		**24 hours, pooled at 8 weeks (n=10)**		**24 hours, pooled at 4-6 months**				**Pooled banked milk at >1 month**		**Milk at 21 days –2 months**				
									Mexico (n=40)		**Houston (n=40)**								
mg amino acid per	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g nitro-gen**	**g pro-tein**	**100 kcal**
Cysteine	111	*32*	173	*50*	108	*31*	101	*29*	167	*48*	134	*39*	133	*38*	118	*34*	131	21	*38*
Histidine	111	*32*	156	*45*	255	*73*	112	*32*	112	*32*	108	*31*	122	*35*	150	*43*	141	23	*41*

	Lönnerdal &Forsum (1985)		**Darragh & Moughan (1998)**		**Bindels & Harzer (1985)**		**Janas et al. (1987)**		**Villalpando et al. (1998)**				**Räihä et al. (2002) mod Nayman et al. (1979)**		**Yonekubo et al. (1991)**		**Mean of all amino acids contents**		
	Pooled banked milk at 4-16 weeks		**Pooled over 20 days at 10-14 weeks (n=20)**		**24 hours, pooled at 5 weeks (n=10)**		**24 hours, pooled at 8 weeks (n=10)**		**24 hours, pooled at 4-6 months**				**Pooled banked milk at >1 month**		**Milk at 21 days –2 months**				
									Mexico (n=40)		**Houston (n=40)**								
mg amino acid per	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g nitro-gen**	**g pro-tein**	**100 kcal**
Isoleucine	242	70	333	96	376	108	306	88	292	84	331	95	300	86	374	108	319	51	92
Leucine	457	*132*	598	*172*	713	*205*	611	*176*	528	*152*	541	*156*	572	*165*	667	*192*	586	94	*169*
Lysine	314	*90*	406	*117*	522	*150*	365	*105*	366	*105*	408	*118*	361	*104*	421	*121*	395	63	*114*
Methionine	78	*22*	90	*26*	89	*26*	73	*21*	99	*29*	76	*22*	83	*24*	92	*26*	85	14	*24*
Phenyl-alanine	153	*44*	243	*70*	344	*99*	183	*53*	440	*127*	439	*126*	217	*62*	240	*69*	282	45	*81*
Threonine	217	*62*	316	*91*	344	*99*	251	*72*	248	*71*	242	*70*	256	*74*	269	*77*	268	43	*77*
Tryptophan	NA		NA		172	*50*	79	*23*	112	*32*	89	*26*	111	*32*	122	*35*	114	18	*33*
Tyrosine	201	*58*	241	*69*	369	*106*	191	*55*	292	*84*	299	*86*	233	*67*	249	*72*	259	42	*75*

	Lönnerdal &Forsum (1985)		**Darragh & Moughan (1998)**		**Bindels & Harzer (1985)**		**Janas et al. (1987)**		**Villalpando et al. (1998)**				**Räihä et al. (2002) mod Nayman et al. (1979)**		**Yonekubo et al. (1991)**		**Mean of all amino acids contents**		
	Pooled banked milk at 4-16 weeks		**Pooled over 20 days at 10-14 weeks (n=20)**		**24 hours, pooled at 5 weeks (n=10)**		**24 hours, pooled at 8 weeks (n=10)**		**24 hours, pooled at 4-6 months**				**Pooled banked milk at >1 month**		**Milk at 21 days –2 months**				
									Mexico (n=40)		**Houston (n=40)**								
mg amino acid per	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g N**	**100 kcal**	**g nitro-gen**	**g pro-tein**	**100 kcal**
Valine	253	*73*	327	*94*	376	*108*	267	*77*	286	*82*	331	*95*	317	*91*	364	*105*	315	50	*90*

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Annex II

GENERAL PRINCIPLES FOR ESTABLISHING MINIMUM AND MAXIMUM VALUES FOR THE ESSENTIAL COMPOSITION OF INFANT FORMULA

1. The goal of establishing minimum and maximum values is to provide safe and nutritionally adequate infant formula products that meet the normal nutritional requirements of infants.

2. A nutritionally adequate infant formula will promote growth and development consistent with science based standards and meet the nutritional requirements of infants when fed as a sole source of nutrition during the first months of life up to the introduction of appropriate complementary feeding.

3. The values to be established are based on an independent evaluation, in particular of the scientific evidence of the amounts needed to meet the nutritional requirements of infants, considering relevant human infant studies and the composition of breast-milk.

4. In addition to the principles set out in No. 3, when setting minimum and maximum values, consideration will also be given to the safety of such values.

For nutrients with a documented risk of adverse health effects the upper levels to be taken into account will be determined using a science-based risk assessment approach. Where scientific data are not sufficient for a science-based risk assessment, consideration should be given to an established history of apparently safe use of the nutrient in infants, as appropriate. Values derived on the basis of meeting the nutritional requirements of infants and an established history of apparently safe use should be considered as interim guidance upper levels. The approach to setting maximum and upper guidance values shall be made transparent and comprehensible.

5. When establishing minimum and maximum amounts, the following should also be taken into account:

a) bioavailability, processing losses and shelf-life stability from the ingredients and formula matrix,

b) total levels of a nutrient in infant formula, taking into account both naturally occurring nutrients in the ingredients and added nutrients,

c) the inherent variability of nutrients in ingredients and in water that may be added to the infant formula during manufacture.

6. Overages for individual nutrients, as appropriate, to ensure that the required minimum levels are met throughout the shelf-life of the formula, will be included in the maximum value.

7. In establishing minimum or maximum amounts of nutrients per 100 kcal (or per 100 kJ) of infant formula based on consideration of reference values for the nutrients expressed as units per daily intake or per kilogram of body weight, the following assumptions will be considered:

a) The mean intake of prepared formula for infants from birth to six months of age is 750 ml per day, and

b) a representative body weight for an infant over this period is 5 kg,

and

c) a representative caloric intake of an infant over this period is 500 kcal per day (or 100 kcal/kg/day).

Modifications of the approach may be needed when there is justification for deviating from one or more of these assumptions with regard to the specific formula product or specific infant population group.

SECTION B: FORMULA FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS

1. SCOPE

1.1 This section of the Standard applies to Formula for Special Medical Purposes Intended for Infants in liquid or powdered form intended for use, where necessary, as a substitute for human milk or infant formula in meeting the special nutritional requirements arising from the disorder, disease or medical condition for whose dietary management the product has been formulated.

1.2 This section of the Standard contains compositional, quality, labelling and safety requirements for Formula for Special Medical Purposes Intended for Infants.

1.3 Only products that comply with the criteria laid down in the provisions of this section of this standard would be accepted for marketing as formula for special medical purposes intended for infants.

1.4 The application of this section of the Standard should take into account, as appropriate for the products to which the section applies and the special needs of the infants for whom they are intended, the recommendations made in the International Code of Marketing of Breast-milk Substitutes (1981), the Global Strategy for Infant and Young Child Feeding and World Health Assembly resolution WHA54.2 (2001).

2. DESCRIPTION

2.1 Product definition

2.1.1 Formula for Special Medical Purposes Intended for Infants means a substitute for human milk or infant formula that complies with Section 2, Description, of the Codex Standard for the Labelling of and Claims for Foods for Special Medical Purposes (CODEX STAN 180-1991) and is specially manufactured to satisfy, by itself, the special nutritional requirements of infants with specific disorders, diseases or medical conditions during the first months of life up to the introduction of appropriate complementary feeding.

2.1.2

See Section A 2.1.2

2.2 Other Definitions

See Section A 2.2

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Essential Composition

3.1.1. Formula for Special Medical Purposes intended for Infants is a product based on ingredients based of animal, plant and/or synthetic origin suitable for human consumption. All ingredients and food additives shall be gluten-free.

3.1.2 The composition of Formula for Special Medical Purposes Intended for Infants shall be based on sound medical and nutritional principles. The nutritional safety and adequacy of the formula shall be scientifically demonstrated to support growth and development in the infants for whom it is intended,

as appropriate for the specific products and indications. Their use shall be demonstrated by scientific evidence to be beneficial in the dietary management of the infants for whom it is intended.

3.1.3 The energy content and nutrient composition of Formula for Special Medical Purposes intended for infants shall be based on the requirements for infant formula as given in sections A 3.1.2 and A 3.1.3, except for the compositional provisions which must be modified to meet the special nutritional requirements arising from the disease(s), disorder(s) or medical condition(s) for whose dietary management the product is specifically formulated, labelled and presented.

3.1.4 In addition to the requirements in 3.1.3 the following requirements shall also be taken into account, where appropriate:

Chromium

Unit	**Minimum**	**Maximum**	**GUL**
µg/100 kcal	1.5	-	10
µg/100 kJ	0.4	-	2.4

Molybdenum

Unit	**Minimum**	**Maximum**	**GUL**
µg/100 kcal	1.5	-	10
µg/100 kJ	0.4	-	2.4

3.2 Optional ingredients

3.2.1 In addition to the compositional requirements listed under 3.1.3, other ingredients may be added in order to provide substances ordinarily found in human milk or required to ensure that the formulation is suitable as the sole source of nutrition for the infant and for the dietary management of his/her disease, disorder or medical condition.

3.2.2 The suitability for the intended special medical purpose, the suitability for the particular nutritional use of infants and the safety of these substances shall be scientifically demonstrated. The formula shall contain sufficient amounts of these substances to achieve the intended effect.

3.2.3 Only L(+)lactic acid producing cultures may be used in Formulas for Special Medical Purposes for infants if shown to be safe and appropriate for use in these vulnerable populations.

3.3 Vitamin Compounds and Mineral Salts

See Section A 3.4

3.4 Consistency and Particle Size

See Section A 3.5

3.5 Purity Requirements

See Section A 3.6

3.6 Specific Prohibitions

See Section A 3.7

4. FOOD ADDITIVES

See Section A 4.

5. CONTAMINANTS

See Section A 5.

6. HYGIENE

See Section A 6.

7. PACKAGING

See Section A 7.

8. FILL OF CONTAINER

See Section A 8.

9. LABELLING

See introductory paragraph of Section A 9.

9.1 The Name of the Food

9.1.1 See Section A 9.1.1

9.1.2 The name of the product shall be "Formula for Special Medical Purposes Intended for Infants" or any appropriate designation indicating the true nature of the product, in accordance with national usage.

9.1.3 If cows' milk is the only source of protein, the product may be labelled "Formula for Special Medical Purposes Intended for Infants Based on Cows' Milk".

9.2 List of Ingredients

See Section A 9.2

9.3 Declaration of Nutritive Value

Formula for Special Medical Purposes Intended for Infants shall be labelled with complete nutrition labelling according to Section 4.2 of Codex Standard for the Labelling of and Claims for Foods for Special Medical Purposes (CODEX STAN 180-1991).

9.4 Date Marking and Storage Instructions

See Section A 9.4

9.5 Information for Use

See Section A 9.5

9.6 Additional Labelling Requirements

9.6.1 Formula for Special Medical Purposes Intended for Infants shall be labelled with the additional information as specified in Sections 4.4.1, 4.4.3, 4.4.4, 4.5.1 and 4.5.5 of CODEX STAN 180-1991.

9.6.2 A prominent statement indicating that the product is intended as the sole source of nutrition shall appear on the label.

9.6.3 In addition, the information specified in Sections 4.5.2, 4.5.3 and 4.5.6 of CODEX STAN 180-1991 shall be included on the label or be provided separately from the package.

9.6.4 Labels and information provided separately from the package should not discourage breastfeeding, unless breastfeeding is contraindicated on medical grounds for the disease(s), disorder(s) or medical condition(s) for which the product is intended.

9.6.5

See Section A 9.6.5

10. Methods of Analysis

See Section A 10.

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

MADE IN VERMONT

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:
PROTEIN g	2.5
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 B_1) mcg	80
RIBOFLAVIN (VITAMIN B_2) mcg	90
VITAMIN B_6 mcg	60
VITAMIN B_{12} mcg	0.3
NIACIN mcg	1000
FOLIC ACID (FOLACIN) mcg	16
PANTOTHENIC ACID mcg	500

VITAMINS:	PER 100 CALORIES:
BIOTIN mcg	3
VITAMIN C (ASCORBIC ACID) mg	12
CHOLINE mg	24
INOSITOL mg	6
MINERALS:	
CALCIUM mg	105
PHOSPHORUS mg	69
MAGNESIUM mg	11
IRON mg	1.8
ZINC mg	1.2
MANGANESE mcg	25
COPPER mcg	75
IODINE mcg	15
SELENIUM mcg	2.8
SODIUM mg	36
POTASSIUM mg	120
CHLORIDE mg	80

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)
Ⓟ 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.

6 83744 13002 0

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

MATERIAL SAFETY DATA SHEET

L-METHIONINE USP/FCC

Material no.		Version	**2.8 / US**
Specification	**140323**	Revision date	**08/27/2011**
Order Number		Print Date	**09/28/2011**
		Page	**1 / 7**

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

For Food, Drug or Cosmetic Use Only

Product information

Trade name	:	L-METHIONINE USP/FCC
Use of the Substance / Preparation	:	Pharmaceutical intermediate
Company	:	Evonik Degussa Corporation USA 379 Interpace Parkway Parsippany,NJ 07054 USA
Telephone	:	973-541-8000
Telefax	:	973-541-8040
US: CHEMTREC EMERGENCY NUMBER	:	800-424-9300
CANADA: CANUTEC EMERGENCY NUMBER	:	613-996-6666
Product Regulatory Services	:	973-541-8060

2. HAZARDS IDENTIFICATION

*** EMERGENCY OVERVIEW ***

***Form**-crystalline* ***Color**-white* **Odor**-*Mild, characteristic odor.*

Dust may be irritating to respiratory tract.
Fine dust, which may be formed through abrasion during transport or handling, can form explosive mixtures with air

POTENTIAL HEALTH EFFECTS

Eye contact

Possibly irritating.

Skin Contact

Not expected to be absorbed through skin.

Inhalation

May cause irritation to the respiratory tract.

Ingestion

Regarded as essentially non-toxic by ingestion.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Other information

This product does not contain any components considered to be health hazards under the OSHA Hazard Communication Standard 29 CFR 1910.1200 or under the WHMIS Controlled Product Regulations in Canada.

4. FIRST AID MEASURES

Inhalation

If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If unconscious, evaluate the need for artificial respiration. Get immediate medical attention.

Skin contact

Wash with water and soap as a precaution.

Eye contact

In case of contact, immediately flush eyes with plenty of water. Obtain medical attention if irritation develops.

Ingestion

If swallowed, rinse mouth with water, then drink large quantities of water to rinse throat and dilute stomach contents. Never give anything by mouth to an unconcious person.
Consult a physician immediately.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media

Use water spray or fog, foam, dry chemical or CO2.

Specific hazards during fire fighting

In the case of fire, the following hazardous smoke fumes may be produced: flammable smouldering gases nitrogen oxides (NOx) Sulphur oxides In the event of fire and/or explosion do not breathe fumes.

Special protective equipment for fire-fighters

As in any fire, wear self-contained positive-pressure breathing apparatus, (MSHA/NIOSH approved or equivalent) and full protective gear.

Further information

Avoid dust formation.

6. ACCIDENTAL RELEASE MEASURES

Environmental precautions

Obey relevant local, state, provincial and federal laws and regulations. Do not contaminate any lakes, streams, ponds, groundwater or soil.

Methods for cleaning up

Collect material and place in a disposal container.

Use cleaning techniques that do not generate dust clouds if ignition sources are present.

Dusts can form explosive mixtures with air.

Use only vacuum cleaners approved for combustible dust collection.

Additional advice

If dust is present, control smoking, open flames, sparks, static electricity and friction heat.

7. HANDLING AND STORAGE

Handling

Safe handling advice

Minimize dust generation and accumulation.
May form flammable dust-air mixtures.
Avoid breathing dust.

Advice on protection against fire and explosion

Prevent the generation of dust clouds, since dusts can form explosive mixtures with air. If dust forms, remove all sources of ignition and static discharge.

Do not allow dust to collect in open or hidden areas.
In product transfer systems involving the use of air as a fluidizing medium, the user must be sure to dissipate static charge by careful bonding and grounding of all equipment and personnel involved in fluid transfer, with continuity checks to prove effectiveness.

Additional guidance on fire and explosion protection may be found in the consensus standard NFPA 654 for chemical dusts.

Storage

Requirements for storage areas and containers

Keep away from heat. Store in a cool, dry place. Keep container closed when not in use.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Component occupational exposure guidelines

- **exposure limit for dust**

CAS-No.		
Control parameters	15 mg/m3	Time Weighted Average (TWA) Permissible Exposure Limit (PEL):(OSHA Z1)
	Total dust.	
	5 mg/m3	Time Weighted Average (TWA) Permissible Exposure Limit (PEL):(OSHA Z1)
	Respirable fraction.	

10 mg/m3 Time Weighted Average (TWA):(ACGIH)
Inhalable fraction.

3 mg/m3 Time Weighted Average (TWA):(ACGIH)
Respirable fraction.

Engineering measures

Avoid dust formation and control ignition sources. Employ grounding, venting and explosion relief provisions in accordance with accepted engineering practices in any process capable of generating dust and/or static electricity.

To identify additional system design issues with respect to dust hazards, it is recommended to conduct a dust hazard analysis using information and sources provided in the OSHA Fact Sheet on combustible dusts (DSG 3/2008) and addressing enforcement issues identified in the Combustible Dust National Emphasis Program (Reissued) (CPL 03-00-008, 3/11/08)

Personal protective equipment

Respiratory protection

A respiratory protection program that meets OSHA 1910.134 and ANSI Z88.2 or applicable federal/provincial requirements must be followed whenever workplace conditions warrant respirator use. NIOSH's "Respirator Decision Logic" may be useful in determining the suitability of various types of respirators.

Hand protection

Use impermeable gloves.

Eye protection

Wear safety glasses with side shields.

Skin and body protection

A safety shower and eye wash fountain should be readily available.
To identify additional Personal Protective Equipment (PPE) requirements, it is recommended that a hazard assessment in accordance with the OSHA PPE Standard (29CFR1910.132) be conducted before using this product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	crystalline
Color	white
Odor	Mild, characteristic odor.

Safety data

pH	5.6 - 6.1
Melting point/range	276 - 279 °C
Explosiveness	Dust, which can occur through abrasion, can combine with air to form a mixture which can be explosive.

Bulk density	420 kg/m3

10. STABILITY AND REACTIVITY

Conditions to avoid	Operations that create dust.
Hazardous decomposition products	Sulphur oxides, nitrogen oxides (NOx), Carbon oxides
Thermal decomposition	Stable under normal conditions.

11. TOXICOLOGICAL INFORMATION

Product Acute oral toxicity	LD50 Rat: > 10000 mg/kg (literature)

12. ECOLOGICAL INFORMATION

General Ecological Information	There are no ecological data available.

13. DISPOSAL CONSIDERATIONS

WASTE DISPOSAL

Advice on disposal	Waste must be disposed of in accordance with federal, state, provincial and local regulations.

14. TRANSPORT INFORMATION

Transport/further information
Not dangerous according to transport regulations.

15. REGULATORY INFORMATION

Information on ingredients / Non-hazardous components

This product contains the following non-hazardous components

L-Methionine
CAS-No. 63-68-3 Percent (Wt./ Wt.) 100 %

US Federal Regulations

OSHA

If listed below, chemical specific standards apply to the product or components:

- None listed

Clean Air Act Section (112)

If listed below, components present at or above the de minimus level are hazardous air pollutants:

- None listed

CERCLA Reportable Quantities

If listed below, a reportable quantity (RQ) applies to the product based on the percent of the named component:

- None listed

SARA Title III Section 311/312 Hazard Categories

The product meets the criteria only for the listed hazard classes:

- No SARA Hazards

SARA Title III Section 313 Reportable Substances

If listed below, components are subject to the reporting requirements of Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 and 40 CFR Part 372:

- None listed

Toxic Substances Control Act (TSCA)

If listed below, non-proprietary substances are subject to export notification under Section 12 (b) of TSCA:

- None listed

State Regulations

California Proposition 65

A warning under the California Drinking Water Act is required only if listed below:

- None listed

International Chemical Inventory Status

Unless otherwise noted, this product is in compliance with the inventory listing of the countries shown below. For information on listing for countries not shown, contact the Product Regulatory Services Department.

• Europe (EINECS/ELINCS)	Listed/registered
• USA (TSCA)	Regulated food, drug, cosmetic
• Canada (DSL)	Listed/registered
• Australia (AICS)	Listed/registered
• Japan (MITI)	Listed/registered
• Korea (TCCL)	Listed/registered
• Philippines (PICCS)	Listed/registered
• China	Listed/registered

16. OTHER INFORMATION

HMIS Ratings

Health :	0
Flammability :	N
Physical Hazard :	0

Further information

Changes since the last version are highlighted in the margin. This version replaces all previous versions.

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

A limitation of these clinical studies is that they were done in humans with a disease. Also, the longest study was only 6 months. Finally, only a limited number of endpoints were investigated. McCune and coworkers (1984) reported no effects on plasma sodium, potassium, and chloride in 41 patients treated for 24 weeks with 1,248 mg/d of L-lysine monohydrochloride.

Dose–Response Assessment

As mentioned above, very few adverse effects of L-lysine have been observed in humans or animals after high, mostly acute, doses. Thus, the data on the adverse effects of L-lysine from supplements were considered not sufficient for a dose–response assessment and derivation of a UL for apparently healthy humans.

Methionine

L-Methionine is an indispensable amino acid with glycogenic properties. In animal studies, it has been described as one of the more toxic amino acids (Health and Welfare Canada, 1990). Humans, as well as other mammals, cannot fix inorganic sulfur into organic molecules and must rely on ingested sulfur amino acids, such as methionine, for the synthesis of protein and biologically active sulfur. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of methionine from food and supplements is 1.8 g/d (Appendix Table D-12). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 4.1 g/d.

Hazard Identification

Adverse Effects in Animals. Dietary excesses of L-methionine (2.7 percent of the diet) for 6, 13, or 20 days have been associated with erythrocyte engorgement and accumulation of hemosiderine in rats (Benevenga et al., 1976), and there was a depression of growth and splenic damage. A single dietary dose (2.7 percent of the diet) of L-methionine decreased body growth and also reduced food intake in rats (Steele et al., 1979).

Dietary intakes of 2 to 4 percent of L-methionine caused slight changes in liver cells in rats (Stekol and Szaran, 1962) and slight decreases in liver iron content (Klavins et al., 1963). Darkened spleens caused by increases in iron deposition have been observed in weanling rats fed 1.8 percent methionine diets for 28 days (Celander and George, 1963).

Viau and Leathem (1973) fed pregnant rats 4 percent of their diet as methionine and reported subnormal fetal and placental weights. However, supplemental methionine prevented neural tube defects in rat embryos treated with teratogenic antivisceral yolk sac serum (Fawcett et al., 2000). In the mouse, the administration of methionine reduced experimentally induced spina bifida (Ehlers et al., 1994). Other studies in rodent and primate models support the beneficial effect of methionine supplementation in improving pregnancy outcomes (Chambers et al., 1995; Chatot et al., 1984; Coelho and Klein, 1990; Ferrari et al., 1994; Moephuli et al., 1997).

Adverse Effects in Humans. Single oral doses of about 0.6 g (adults) and 0.08 g (infants) led to increased plasma levels of L-methionine and L-alanine, and decreased plasma concentrations of leucine, isoleucine, valine, tyrosine, tryptophan, and phenylalanine (Stegink et al., 1980, 1982b). Neither report included mention of any adverse effects. Methionine supplements (5 g/d) for periods of weeks were reportedly innocuous in humans (Health and Welfare Canada, 1990). A single oral dose of 7 g has been associated with increased plasma concentrations of methionine and the presence of mixed sulfides (Brattstrom et al., 1984). Single oral doses of 7 g produced lethargy in six individuals and oral administration of 10.5 g of L-methionine to one produced nausea and vomiting (Perry et al., 1965). After an oral administration of 8 g/d of methionine (isomer not specified) for 4 days, serum folate concentrations were decreased in five otherwise healthy adults (Connor et al., 1978).

High doses of methionine (~100 mg/kg of body weight) led to elevated plasma methionine and homocysteine concentrations (Brattstrom et al., 1984, 1990; Clarke et al., 1991; Wilcken et al., 1983). Thus, it was concluded that elevated plasma homocysteine concentrations may be a risk factor for coronary disease (Clarke et al., 1991).

Infants more rapidly metabolized methionine than adults (Stegink et al., 1982b). In women whose average daily intake of methionine was above the lowest quartile of intake (greater than 1.34 g/d), a 30 to 40 percent reduction in neural tube defect-affected pregnancies was observed (Shaw et al., 1997). These reductions were observed for both anencephaly and spina bifida.

Dose–Response Assessment

There are no adequate data to characterize a dose–response relationship for L-methionine. Thus the data on the adverse effects of L-methionine from supplements were considered not sufficient for a dose–response assessment and derivation of a UL for apparently healthy humans.

Methionine fortification of a soy protein formula fed to infants[1, 2]

Samuel J. Fomon,[3] M.D., Ekhard E. Ziegler,[4] M.D., Lloyd J. Filer, Jr.,[3] M.D., Ph.D., Steven E. Nelson,[5] B.S., and Barbara B. Edwards,[6] R.N.

ABSTRACT Data from study of nine normal full-term infants fed a soy isolate-based formula unsupplemented with methionine were compared with similar data from study of 10 similar infants fed the same formula supplemented with L-methionine and with data from previous studies of larger groups of infants receiving various other feedings. Food intake, growth, and serum chemical values were studied from 8 through 111 days of age. In addition, nitrogen balance studies were carried out. Statistically significant differences were as follows: lesser weight gain per 100 kcal by infants fed the unsupplemented soy isolate-based formula than by infants fed milk-based or other soy isolate-based formulas; lesser serum concentrations of albumin at age 28 days by infants fed the unsupplemented soy isolate-based formula than by breast-fed infants; greater serum concentrations of urea nitrogen by infants receiving the unsupplemented soy isolate-based formula than by those receiving the same formula supplemented with L-methionine. A number of other differences was noted but were not statistically significant. The results suggest that normal infants fed a formula providing 2.25 g/100 kcal of a soy protein isolate not fortified with methionine performed less well during the first 6 weeks of life than did breast-fed infants and infants fed milk-based formulas or other soy isolate-based formulas fortified with methionine. The limiting nutrient appears to have been methionine. *Am. J. Clin. Nutr.* 32: 2460–2471, 1979.

In considering nutritional properties of soy protein isolates, studies of infants are of particular interest. It has been estimated (1) that approximately 10% of infants in the United States are fed soy isolate-based formulas during the early months of life. This represents the only large group of subjects likely to obtain all or nearly all of their protein intake from this source over extended periods of time.

The biological quality of soy protein isolates, as determined by rat assay, ranges approximately from 65 to 85% that of casein (2). Addition of the first limiting amino acid, methionine, improves quality to that of casein in rat assays (3) and to about 85% that of casein when assayed with newborn pigs (4). Proteins with biological quality less than 70% that of casein are prohibited from use in infant formulas in the United States (5). Infant formulas with protein from soy protein isolate are therefore regularly supplemented with L-methionine. However, few studies of the nutritional effect of methionine fortification of soy proteins have been carried out with human infants. Graham (6) performed nitrogen balance studies with four subjects, 6 to 21 months old, during recovery from malnutrition. With diets providing 4 or 6.4% of energy from soy protein isolate, all four subjects showed improved nitrogen balance when a supplement of 20 mg/kg per day of DL-methionine was given. The nature of the soy protein isolate employed by Graham (6) was not stated.

We have reported (7) that female infants fed a formula providing 6.5% of energy from a soy protein isolate fortified with L-methionine demonstrated growth and serum chemical parameters indistinguishable from those of breastfed infants studied in similar fashion (8). Methionine intake was 24 mg/100 kcal and it was concluded that the requirement of normal infants for methionine was probably not greater than that value. It seemed likely that a formula providing a similar amount of

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methionine (per 100 kcal) exclusively from soy protein isolate, rather than from soy protein isolate plus added L-methionine, would also satisfy the requirement of normal infants for methionine.

The present study provides observations of food intake and growth of normal infants 8 to 112 days old fed a formula in which 9% of energy was derived from soy protein isolate. This formula provided 25.9 mg/100 kcal of methionine, all from soy protein isolate. It had been our intention to feed this formula to a group of 15 normal infants and to compare their growth and serum chemical parameters with those of previously studied breastfed infants (8) and infants fed various formulas (7, 9; S. J. Fomon et al., unpublished data). Subsequent events led us to change the experimental design so that one group of infants received the formula with and another group without a supplement of methionine. Thus, the present study provides observations of the effect of methionine fortification of soy protein isolate used in infant feeding. In addition to observations of food intake and growth, metabolic balance studies were carried out.

Materials and methods

Subjects and enrollment procedures

Twenty-one normal full-term male infants with birth weights more than 2500 g served as subjects in the study of food intake and growth. They were enrolled between 6 and 9 days of age and all but one were observed until 112 days of age. Other normal infants (two males and three females), ranging from 67 to 307 days old at the time of study, served as subjects for metabolic balance studies. The majority of the infants was children of students or faculty of the University of Iowa.

At the time an infant was enrolled in the study, his mother was interviewed by one of us (B.B.E.). The program was outlined in detail, written permission was obtained, and written instructions were provided. The protocol for the study was reviewed and approved by the Committee on Research Involving Human Subjects of the University of Iowa.

As already mentioned, our intention had been to study 15 infants fed the experimental formula (formula I-501) unfortified with methionine. The first three infants (subjects 2221, 2222, and 2223—see Appendix Table 1) were enrolled within a few days of one another and the fourth infant (subject 2224) was enrolled 9 weeks later. The first three infants demonstrated normal rates of growth; serum concentrations of albumin at age 28 days ranged from 3.05 to 3.15 g/dl. However, at 56 days of age two of the three infants demonstrated serum concentrations of albumin less than 3.00 g/dl. We considered these values suspiciously low and the infants were, therefore, maintained under close observation and enrollment of additional infants was temporarily suspended.

At age 84 days serum concentrations of albumin of two infants (blood was not obtained from the third infant) were completely satisfactory: 3.65 and 3.78 g/dl. Enrollment was, therefore, resumed and the next four infants enrolled were given the formula with a supplement of L-methionine in the form of drops. Because further observation of the first four infants enrolled (those without methionine supplementation) offered no cause for concern regarding growth or serum concentrations of albumin, infants were thereafter enrolled alternately with or without the methionine supplement. The available supply of formula limited the study to 21 infants.

Of the infants who served as subjects in balance studies, one (subject 2262, birth weight 3630 g) had served as subject for a study of food intake and growth during the interval 8 to 112 days of age and was fed a milk-based formula during that time. This infant had thus not previously served as subject for metabolic balance studies. The other four infants (subjects 2151, 2179, 2159, and 2281—birth weights 2905, 3090, 3330, and 3245 g, respectively) were enrolled as subjects for metabolic balance studies during the first 2 weeks of life and participated in metabolic balance studies at approximately 14-day intervals until age 112 days, and at 14- to 28-day intervals thereafter. Before study with formula I-501, subject 2281 had been fed a formula containing partially demineralized whey and subjects 2151, 2179, and 2159 had been fed milk-based formulas.

Feeding

During the first few days after birth until 6 to 9 days of age nearly all of the infants who were to be enrolled in the growth study were fed commercially prepared milk-based formulas. Subsequently, experimental formula I-501 was fed ad libitum. This formula was prepared from soy protein isolate (Edi-Pro A, Ralston Purina Company), vegetable oils (58% corn oil, 42% coconut oil), carbohydrate (70% corn syrup solids, 30% sucrose), vitamins, and minerals. Data on chemical composition of the formula are presented in Table 1. Expressed per 100 kcal, the formula provided 2.25 g of protein, 25.9 mg of methionine, and 27.0 mg of cystine. The formula was prepared by the manufacturer (Ross Laboratories, Columbus, Ohio) as a single batch and was supplied in 11,000 ready-to-feed, disposable glass bottles containing 240 ml. Formula was delivered to the family and volume of intake determined as previously described (7, 9).

The infants were fed this formula with or without a supplement of L-methionine. The supplement provided 12 mg of L-methionine per milliliter of distilled water and approximately 1 ml of this solution was added to 240 ml of formula immediately before feeding. The addition was made by adding 25 drops of L-methionine solution to the bottle (subjects 2225, 2227, 2228, and 2229) or by adding the amount by means of a dropper calibrated to deliver 1 ml. With the supplement added, methionine content of the formula became 22.6 mg/dl (33.2 mg/100 kcal).

During the first 28 days of life the formula and a fluoride supplement (Karidium Liquid, Lorvic Corpo-

TABLE 1
Composition of formula I-501

Energy (kcal/100 ml)	68
Major constituents (g/100 ml)	
Protein	1.53
Fat	3.68
Carbohydrate	7.20
Mineral content per liter	
Calcium (mg)	644
Phosphorus (mg)	407
Sodium (mEq)	14.5
Potassium (mEq)	16.8
Chloride (mEq)	15.5
Magnesium (mg)	51
Iron (mg)	16.1
Zinc (mg)	6.0
Copper (mg)	1.27
Vitamin content per liter	
Vitamin A (IU)	2900
Vitamin D (IU)	400
Vitamin E (IU)	15
Vitamin K (mg)	0.15
Thiamin (mg)	0.39
Riboflavin (mg)	0.78
Niacin (mg)	11.8
Pyridoxine (mg)	0.66
Folic acid (mg)	0.1
Pantothenic acid (mg)	5
Ascorbic acid (mg)	120
Biotin (mg)	0.15
Choline (mg)	60
Sulfur-containing amino acids (mg/g protein)	
Methionine	11.5[a]
Cystine	12.0[a]

[a] Typical values for EdiPro A (F. H. Steinke, personal communication, 1978).

ration, St. Louis, Mo., 0.25 ml daily) (0.5 mg of fluoride from sodium fluoride) served as the sole source of nutrients. After 28 days of age, the infants were permitted to receive, in addition to the above, commercially prepared beikost (foods other than formula or milk) from one manufacturer (Gerber Products Company, Fremont, Mich.) according to the following schedule: from age 28 days, oatmeal with applesauce and bananas; from 56 days, pears; and from 84 days (two foods), applesauce, and bananas with tapioca. The proximate composition and density of each strained food has been presented previously (9). Caloric densities of the four foods were 75, 69, 81, and 88 kcal/100 g, respectively. Parents were advised that addition of such foods to the diet was optional and that the formula was a complete food. Empty (or partially empty) jars of strained food were collected at 3- or 4-day intervals and weighed. From the weight of strained food consumed and its density, volume of intake of each of these strained foods was calculated. For reasons discussed previously (10), wastage of strained foods (amounts remaining in feeding dish, wiped from infant's face, etc.) were considered relatively unimportant in estimating total food intake.

Procedures

Body weight was determined to the nearest 5 g as described previously (7–9) and length was determined as described by Fomon (11). Measurements were made between 6 and 9 days of age, within 2 days of each of the following ages: 14, 28, 42, and 56 days; and within 4 days of ages 86 and 112 days. A detailed description of the intervals of study and of the data analysis has been presented previously (7–9). Venous blood was obtained as previously described (7–9).

Metabolic balance studies of 72 hr duration were carried out in the manner previously described (11). Each subject had at least one balance study (subjects 2281 and 2262 had two balance studies) performed with the unsupplemented formula and one while receiving the methionine supplement. The formula and, where applicable, the methionine supplement were fed for at least 11 days before a balance study.

Methods used in analyses of serum, foods, and excreta were those described previously (7). Plasma concentrations of amino acids were determined with a Technicon NC-1 amino acid analyzer (Technicon Instruments, Tarrytown, N. Y.) using the buffer system of Efron (12).

Results of the present study will be compared with data from other observations of infants studied in similar fashion: "breast-fed infants" refers to 117 male breast-fed infants (13); "infants fed milk-based formulas" refers to 203 male infants fed various formulas based on cow milk (9; S. J. Fomon, unpublished data); and "infants fed soy isolate-based formulas" refers to 63 male infants fed various formulas based on methionine-fortified soy protein isolates (7; S. J. Fomon, unpublished data). These formulas provided approximately 2.1 to 2.6 g of soy protein isolate per 67 kcal and were supplemented with 3.6 to 8.0 mg of methionine per gram of protein.

Results

Detailed data concerning each of the 21 infants enrolled in the study are presented in Appendix Tables 1 to 5. Ten infants were fed the unsupplemented formula and 11 received the supplement of L-methionine. All but one infant (subject 2240; fed the unsupplemented formula) completed the planned period of study to 112 days of age. This infant gained from 3110 to 3540 g (21.5 g/day) between 8 and 28 days of age (Appendix Table 1), a gain slightly greater than the 5th percentile value for breast-fed infants (13). The infant had had diarrhea during the 2 days preceding the 28-day visit and was withdrawn from study on the advice of his physician a day or two after that visit.

One infant (subject 2227) who completed the planned period of observation to 112 days of age had been noted at birth to have a heart murmur and was briefly hospitalized at 98 days of age for treatment of diarrhea. It is apparent from data included in Appendix

TABLE 2
Summary of data on food intake

	Age interval (days)					
	8–41		42–111		8–111	
	mean	*SD*	*mean*	*Sd*	*mean*	*SD*
Formula I-501 unsupplemented—9 infants						
Average body wt (g)	4004	400	5617	577	5090	498
Food consumption						
Formula						
(ml/day)	737	145	792	130	774	127
(kcal/day)	501	99	539	88	526	86
Beikost						
(ml/day)	1	2	67	54	46	36
(kcal/day)	1	2	56	45	38	30
Total						
(ml/day)	739	145	859	99	820	112
(kcal/day)	502	99	594	64	564	74
(kcal/kg/day)	125	20	106	4	113	8
Protein intake						
(g/day)	11.30	2.22	12.83	1.69	12.33	1.81
(g/kg/day)	2.81	0.45	2.30	0.12	2.47	0.19
(g/100 kcal)	2.25	0.00	2.16	0.08	2.18	0.06
Formula I-501 supplemented with methionine—10 infants						
Average body wt (g)	4537	382	6262	627	5698	523
Food consumption						
Formula						
(ml/day)	753	83	847	92	816	72
(kcal/day)	512	56	576	63	555	49
Beikost						
(ml/day)	10	10	72	45	52	31
(kcal/day)	8	8	58	37	42	26
Total						
(ml/day)	763	80	918	119	868	86
(kcal/day)	520	54	634	85	597	60
(kcal/kg/day	115	7	102	9	106	6
Protein intake						
(g/day)	11.67	1.22	13.70	1.65	13.04	1.22
(g/kg/day)	2.58	0.16	2.20	.17	2.33	0.11
(g/100 kcal)	2.24	0.01	2.16	0.05	2.19	0.04

Table 1 that weight gain of this infant was exceedingly slow (more than 3 SD below the mean for breast-fed infants between 8 and 112 days) and that caloric intake was extremely low. The infant subsequently developed congestive cardiac failure and was found to have coarctation of the aorta. The abnormality was corrected surgically at 19 months of age. Data for this infant are included in the Appendix Tables but have been excluded from data analysis. Data analysis, therefore, concerns 19 infants (nine fed the unsupplemented and 10 fed the supplemented formula) who completed the planned 112 days of study.

Subject 2237 developed vomiting on the 25th day of life, was admitted to the hospital and had pyloromyotomy for pyloric stenosis on the 26th day of life. Fluids were administered intravenously for 24 hr. By the 27th day, formula feedings were resumed. The infant was, therefore, permitted to continue in the study.

Intake of formula and beikost

Data concerning volume of formula consumed by each infant during each age interval are presented in Appendix Table 3. Similar data for items of beikost permitted at various ages are presented in Appendix Table 4. Four

infants received some intake of foods other than those included in the protocol. These foods are identified in Appendix Table 5. Data on food intake are summarized in Table 2.

Energy intake

Despite the lesser mean body size of infants in the group receiving the unsupplemented formula (see "Weight and length" section), mean energy intake (kilocalories per day) during the age interval 8 to 41 days was nearly the same for this group as for the group receiving the methionine supplement (Table 2). Therefore, energy intake per unit of body weight by infants receiving the unsupplemented formula (125 kcal/kg per day) was somewhat greater than that by infants receiving the supplement (115 kcal/kg per day) but the difference was not statistically significant ($P = 0.160$). Mean energy intake by 203 previously studied male infants fed milk-based formulas was 118 kcal/kg per day and that by 63 infants fed L-methionine-supplemented soy isolate-based formulas was 120 kcal/kg per day.

In the interval 42 to 111 days, mean energy intake was 106 kcal/kg per day by the infants receiving the unsupplemented formula and 102 kcal/kg per day by those receiving the methionine supplement. Corresponding values for the larger groups of male infants fed milk-based and soy isolate-based formulas were 100 and 105 kcal/kg per day, respectively. These differences in energy intake were not statistically significant. Similarly, differences in energy intakes during the entire age interval 8 to 112 days were not statistically significant.

As may be seen from Table 2, mean energy intake from beikost was relatively low in each age interval.

Intake of protein and methionine

Because intake of protein from beikost accounted for a relatively small percentage of total protein intake, protein intake per 100 kcal (Table 2) differed only slightly, or not at all, from the protein: energy ratio of the formula (2.25 g of protein per 100 kcal). As a consequence of the greater formula consumption per unit of body weight by infants fed the unsupplemented than by those receiving the supplemented formula, intake of protein per unit of body weight was also somewhat greater by infants fed the unsupplemented formula.

For reasons discussed previously (7), it was assumed that the ratio of amino acids: protein in beikost was the same as that of the soy protein isolate (Table 1). Mean methionine intake by infants receiving the unsupplemented formula was 25.9 mg/100 kcal (32.4 mg/kg per day) in the age interval 8 through 41 days and 24.9 mg/100 kcal (26.4 mg/kg per day) in the age interval 42 through 111 days. Corresponding mean intakes of methionine by infants receiving the supplemented formula were 33.0 and 31.6 mg/100 kcal (38.0 and 32.2 mg/kg per day), respectively, in the two age intervals.

The lesser formula consumption per unit of body weight by infants receiving the methionine supplement resulted in a lesser intake of cystine per unit of body weight than by infants receiving the unsupplemented formula, especially during the age interval 8 through 41 days. Therefore, the intake of total sulfur-containing amino acids per unit of body weight was only slightly greater in the methionine supplemented than in the unsupplemented group. Of course, intake of sulfur-containing amino acids expressed per unit of energy intake was greater in the supplemented than in the unsupplemented group (60.0 versus 52.9 mg/100 kcal in the age interval 8 through 41 days and 57.6 versus 50.7 mg/100 kcal in the age interval 42 through 111 days of age).

Weight and length

As already noted, the two groups of infants were different in body size. Mean weight at birth and mean length at age 8 days of infants fed the unsupplemented formula were similar to those of a larger group of breast-fed infants studied previously (13). Mean weight at birth and mean length at age 8 days of infants fed the supplemented formula were greater ($P < 0.01$) than corresponding values for infants fed the unsupplemented formula or for the series of breast-fed infants. The greater weights and lengths of infants fed the supplemented formula persisted throughout the study (Table 2).

Mean daily gains in weight and length and

gains in weight per unit of energy intake are indicated in Table 3. Included in Table 3 are corresponding data for breast-fed infants and infants fed milk-based and soy isolated-based formulas. During each age interval mean daily weight gain by infants fed the unsupplemented formula was less than that by infants receiving the supplemented formula. Furthermore, mean weight gain of the unsupplemented group was less than that of all other groups of infants studied previously with the exception of breast-fed infants in the age interval 42 to 112 days. None of the differences was statistically significant.

Mean weight gain per unit of energy intake was less by infants fed the unsupplemented formula than by infants fed the supplemented formula, but the difference was not statistically significant. Because birth weight is inversely related to rate of gain in weight during early infancy, the relatively high birth weights of the infants receiving the supplemented formula may have diminished the apparent effect of methionine supplementation. During the age interval 8 to 42 days gain in weight per unit of energy intake by infants fed the unsupplemented formula was significantly less ($P < 0.05$) than that by previously studied infants fed milk-based or soy isolate-based formulas (Table 3), and during the interval 8 to 112 days it was significantly less ($P < 0.05$) than that of infants fed milk-based formulas.

Mean gains in length were quite similar for the two feeding groups and were similar to those for other groups of infants (Table 3). In fact, mean gain in length of infants fed the supplemented formula was significantly greater ($P < 0.05$) than that of the breast-fed infants during the age interval 42 to 112 days.

As may be calculated from data presented elsewhere (13), gains in weight and length of fullterm infants tend to be negatively correlated with size at birth. Therefore, the greater size at birth of infants fed the supplemented formula than of those receiving the unsupplemented formula may be assumed to have had the effect of diminishing differences in rates of gain between the two groups. Analysis of covariance was therefore performed on data from the present study and from our previous study of breast-fed infants (13). However, even when rates of gain in weight and length were corrected for birth weight, differences in

TABLE 3
Summary of data on growth from present study and male infants receiving various feedings

	Age interval (days)					
	8-42		42-112		8-112	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Gain in wt (g/day)						
I-501 unsupplemented (9)[a]	34.2	11.1	26.7	4.1	29.1	5.9
I-501 with methionine (10)	38.0	9.0	29.3	8.8	32.1	6.8
Breastfed (117)[b]	38.7	9.6	25.8	6.1	30.1	5.6
Milk-based formulas (203)[c]	39.5	7.5	28.4	6.3	32.0	5.5
Soy-based formulas (63)[d]	38.7	6.8	29.6	7.0	32.6	5.3
Gain in wt (g/100 kcal)						
I-501 unsupplemented	6.71[e,f]	1.24	4.49	0.53	5.14[e,g]	0.63
I-501 with methionine	7.25	1.42	4.55	0.72	5.35	0.70
Milk-based formulas	8.00[e]	1.20	4.76	0.79	5.70[g]	0.71
Soy-based formulas	7.71[f]	1.26	4.69	0.66	5.55	0.58
Gain in length (mm/day)						
I-501 unsupplemented	1.20	0.22	1.07	0.07	1.11	0.07
I-501 with methionine	1.18	0.32	1.10[h]	0.15	1.12	0.14
Breastfed	1.30	0.23	0.98[h]	0.13	1.09	0.10
Milk-based formulas	1.33	0.20	1.02	0.13	1.12	0.11
Soy-based formulas	1.35	0.21	1.09	0.11	1.18	0.10

[a] Values in parentheses indicate number of infants. [b] Fomon et al. (13). [c] Fomon et al. (9) and unpublished data. [d] Fomon et al. (7) and unpublished data. [e–h] Values with the same superscript differ significantly ($P < 0.05$) from one another.

gains between the unsupplemented and the supplemented groups were not statistically significant.

Blood chemical parameters

As may be seen from Table 4 and Figure 1, mean serum concentrations of urea nitrogen of infants receiving the unsupplemented formula were generally greater than those of infants receiving the supplemented formula. The difference was statistically significant ($P < 0.05$) at each of the four ages.

Although serum concentrations of total protein and albumin of infants receiving the unsupplemented formula tended to be less than those observed in breast-fed infants at all ages, only the albumin concentrations at 28 days of age were significantly (F test, Duncan's multiple range test; $P < 0.05$) less than those of breast-fed infants (Fig. 2). Although slightly higher concentrations were generally observed in infants receiving the supplemented than in those receiving the unsupplemented formula (Table 4), the differences were not statistically significant.

As indicated in Table 4, plasma concentrations of methionine were similar, whereas plasma concentrations of cystine were greater, especially at 56 and 112 days, in infants receiving the supplemented than in those receiving the unsupplemented formula. Because of the lower volumes of intake by infants fed the supplemented formula, intakes of cystine were slightly lower by this group than by infants fed the unsupplemented formula. The greater plasma concentrations of cystine in infants fed the supplemented formula may, therefore, reflect the conversion of methionine to cystine. With the exception of a significantly higher glutamine concentration in the unsupplemented group at 84 days of age, no differences in plasma amino acid concentrations were present. In particular, no statistically significant differences were seen in concentrations of taurine and of total nonessential amino acids.

Metabolic balance studies

As indicated in Table 5, 14 metabolic balance studies were carried out with five infants—seven studies with the unsupplemented formula and seven studies with the methionine-supplemented formula. Each infant received the supplemented and unsupplemented formula on an alternating basis. Mean intakes of nitrogen were almost identical whether the formula was or was not supplemented with methionine. Mean urinary and mean fecal excretions of nitrogen were slightly less and mean retention of nitrogen was slightly greater when the formula was supplemented with methionine than when it was not. However, none of these differences was statistically significant. The

TABLE 4
Serum concentrations of urea nitrogen, total protein, and albumin and plasma concentrations of methionine and cystine

Age	No. of determinations	Urea nitrogen		Total protein		Ablumin		Methionine		Cystine	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
		mg/dl		*g/dl*				*μmole/dl*			
28 days											
I-501 unsupplemented	8	11.5[a]	1.3	5.01	0.51	3.40	0.32	3.4	1.1	3.6	1.1
I-501 with methionine	8	9.5[a]	1.7	5.21	0.38	3.56	0.25	3.3	0.9	4.9	1.5
56 days											
I-501 unsupplemented	8 (9)[b]	10.1[a]	1.3	5.34	0.65	3.53	0.47	3.4	1.3	3.8[c]	0.8
I-501 with methionine		8.6[a]	1.4	5.49	0.23	3.88	0.25	3.2	1.0	5.3[c]	1.1
84 days											
I-501 unsupplemented	7 (6)	10.1[a]	1.7	5.59	0.29	3.95	0.21	3.2	0.7	5.1	0.6
I-501 with methionine	9	8.1[a]	1.3	5.76	0.22	3.92	0.24	2.9	0.7	5.4	1.3
112 days											
I-501 unsupplemented	9 (8)	9.4[a]	1.6	5.62	0.32	4.03	0.26	3.3	1.5	4.5[c]	1.5
I-501 with methionine	8	7.5[a]	1.6	5.90	0.27	4.07	0.20	3.5	0.8	6.6[c]	0.8

[a] Values differ significantly ($P < 0.05$) from each other within the same age group. [b] Nos. in parentheses refer to plasma methionine and cystine where the number of determinations were not the same as for the other determinations. [c] Values differ significantly with $P < 0.01$.

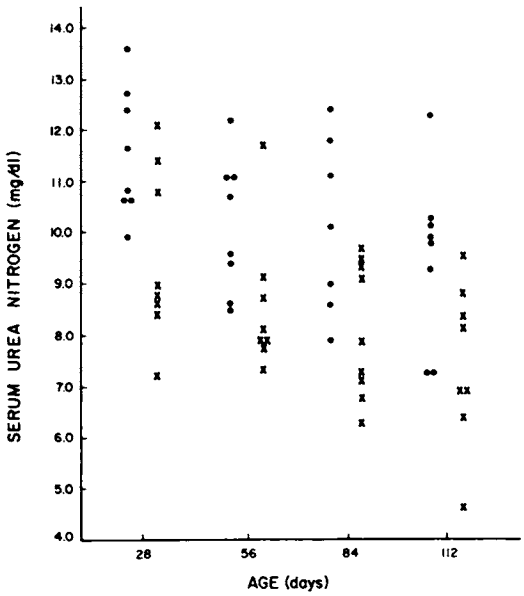

FIG. 1. Serum concentrations of urea nitrogen of infants fed formula I-501 unsupplemented (●) or supplemented with L-methionine (×). At each age the difference was statistically significant (t test, $P < 0.05$).

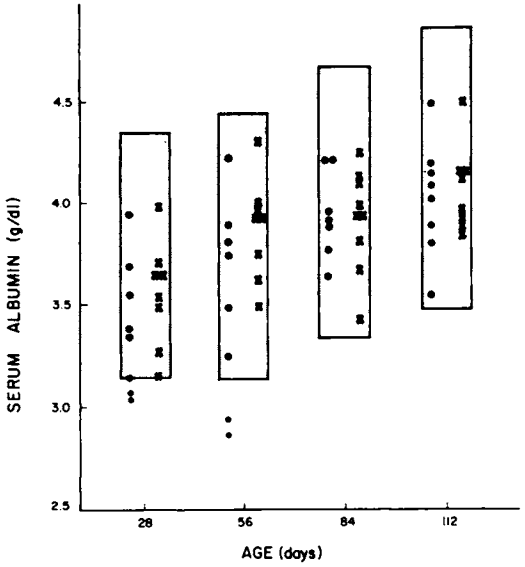

FIG. 2. Serum concentrations of albumin of infants fed formula I-501 unsupplemented (●) or supplemented with L-methionine (×). The *shaded area* indicates values (mean ± 2 SD) of normal breast-fed infants (13). Values of infants fed the unsupplemented formula at 28 days of age are significantly ($P < 0.05$) lower than those of breast-fed infants; all other differences are not statistically significant.

relation of retention of nitrogen to intake of nitrogen (Fig. 3) was similar whether or not the diet was supplemented with methionine.

Discussion

In several other studies of human subjects fed soy proteins, methionine supplementation has been found to improve nitrogen balance. These studies have concerned infants recovering from malnutrition fed a soy protein isolate (6), girls 8 to 9 years old fed soy flour (14), adolescent boys 13 to 17 years old fed a soybean textured product (15), and men fed a soybean textured product (16) or a soy protein isolate (17). Improvement in nitrogen balance with methionine supplementation in these studies was observed only at relatively low intakes of protein. The formula used in the present study provided 2.25 g of protein per 100 kcal, or about 9% of energy intake from protein. This protein intake is well above that of fully breast-fed infants and well above the estimated requirement (approximately 7% of energy in the form of protein) for infants fed high quality protein (11). The present study failed to demonstrate an effect of methionine supplementation on absorption and retention of nitrogen. At this intermediate level of protein intake, protein quality may be better evaluated by longer term observations of weight gain and of serum concentrations of albumin and urea nitrogen than by nitrogen balance studies.

Data from such longer term observations in the present study suggest that normal male infants fed a formula providing the intermediate level of protein intake from the soy protein isolate not supplemented with methionine performed less well, particularly during the first 6 weeks of life, than did previously studied breast-fed infants and infants fed milk-based or methionine-fortified soy isolate-based formulas. Weight gain per unit of energy intake and serum concentrations of albumin were relatively low. Absolute weight gain (grams per day) was also less than that of infants fed other formulas; however, this latter difference was not statistically significant.

The inadequacy of the experimental formula was unanticipated. According to our

TABLE 5
Balances of nitrogen and fat in relation to methionine supplementation

Subject	Age	Weight	Length	Energy intake	Nitrogen				Fat	
					Intake	Excretion		Retention	Intake	Excretion
						Urine	Feces			
	days	*g*	*cm*	*kcal/kg/day*	*mg/kg/day*				*g/kg/day*	
Formula I-501 unsupplemented										
2281	81	6225	59.2	134	483	223	104	156 (32%)	7.27	0.69
	109	6855	60.1	126	454	198	85	170 (38%)	6.84	0.34
2262	138	6740	63.2	97	346	150	69	127 (37%)	4.40	0.18
	180	7530	65.5	85	285	142	59	84 (30%)	3.80	0.35
2179	252	8050	66.6	106	361	180	62	119 (33%)	4.53	0.16
2159	272	6555	64.8	116	397	168	68	161 (41%)	4.91	0.21
2151	293	9250	72.0	85	293	205	63	24 (8%)	3.56	0.47
Mean					374	181	73	120 (31%)	5.04	0.34
SD					76	30	16	52 (11%)	1.45	0.19
Formula I-501 supplemented with L-methionine										
2281	67	5630	56.4	128	461	214	100	147 (32%)	6.94	3.06
	95	6490	59.6	118	426	183	70	173 (41%)	6.41	0.47
2262	124	6610	62.1	112	383	132	48	203 (53%)	5.20	0.17
	152	7005	63.5	89	305	142	58	105 (34%)	3.81	0.15
2179	231	7865	65.9	102	346	158	78	110 (32%)	4.23	0.19
2159	293	6685	66.4	108	368	206	75	87 (24%)	4.85	0.17
2151	307	9320	72.0	115	366	171	63	132 (36%)	4.14	0.15
Mean					380	173	70	137 (36%)	5.08	0.62
SD					51	31	17	41 (9%)	1.19	1.08

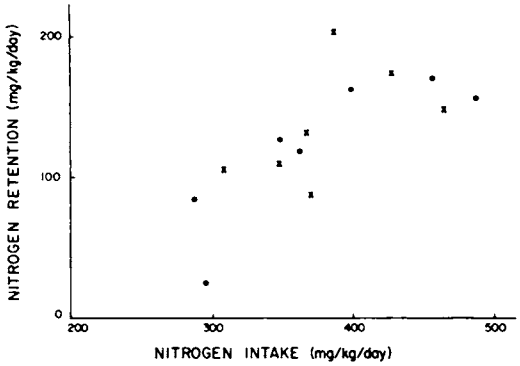

FIG. 3. Retention of nitrogen in relation to intake of nitrogen by five normal infants. Each *point* refers to the result of one 3-day metabolic balance study with formula I-501 unsupplemented (●) or supplemented with L-methionine (×).

calculations[7] the methionine content per 100 kcal of formula was similar to that of a formula studied previously in female infants and judged to be adequate (7). In each case, the soy protein isolate, EdiPro A, was the source of protein. In the earlier study, a formula with relatively low protein content was fortified with L-methionine. In the present study, a formula of greater protein content was unfortified with methionine.

It seems to us unlikely that the differences in results of the two studies reflect either a greater bioavailability of added than of naturally occurring L-methionine or a greater methionine requirement per unit of energy intake for males than for females. It is somewhat more likely that the differences in results of the two studies reflect differences in protein quality of the different batches of EdiPro A or that differences in protein quality resulted from preparation of the formula by the manufacturer.

An additional possibility is that poor performance of the infants fed the soy protein isolate unfortified with methionine resulted from amino acid imbalance. An analogous finding was reported by Wethli et al. (18) in studies of growing chickens. Diets based on corn and soybean meal unsupplemented with methionine gave lesser growth rates than did the same diets supplemented with methionine

[7] Calculation of the methionine content of formula I-501 was based on the assumption that the ratio of methionine to protein was the average ratio reported by the manufacturer. This assumption seems reasonable because analytic errors in determining methionine content may be as great as batch to batch variation.

even though the protein level in the unsupplemented diets was sufficient to meet the assumed methionine requirements. In the case of human infants, it seems likely that such amino acid imbalance is more readily detected by the growth response than by nitrogen balance studies.

Of particular interest in the present study was a definite reduction in concentrations of serum urea nitrogen observed at all ages when the formula was supplemented with methionine. The lesser serum concentrations of urea nitrogen may be interpreted in the light of studies summarized by Bodwell (19). Both in experimental animals and in human subjects, serum concentrations of urea nitrogen are less when proteins of higher quality are fed than when equal intakes of nitrogen are provided from proteins of lesser quality.

Thus, the evidence presented suggests that the quality of protein in the particular soy protein isolate-based formula used in the present study was improved by the addition of L-methionine.

Dr. Lewis D. Stegink, Professor, Departments of Biochemistry and Pediatrics, kindly provided the plasma amino acid analyses.

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Appendix

TABLE 1
Lengths of individual infants fed formula I-501

Subject	Birth date	8	14	28	42	56	84	112
				days				
Unsupplemented								
2221	6.23.75	49.8	50.0	52.1	52.7	54.4	57.8	60.7
2222	6.24.75	49.2	49.8	52.1	52.9	55.5	58.0	60.8
2223	6.25.75	49.9	50.5	52.7	54.6	56.3	58.5	61.7
2224	8.31.75	51.1	51.8	53.8	55.0	55.6	58.9	61.7
2226	1.26.76	52.5	53.4	55.7	57.1	57.9	61.5	65.2
2230	11.23.75	52.0	53.2	55.3	57.2	59.2	61.7	64.2
2233	12.13.75	49.1	49.7	52.6	53.7	56.3	58.8	61.1
2235	1. 3.76	49.8	50.6	52.0	53.2	55.6	58.1	60.8
2238	1.15.76	51.7	51.8	54.0	55.3	57.1	59.7	62.6
2240	2. 9.76	52.8	53.0	54.1				
Supplemented with methionine								
2220	2.25.76	52.0	52.8	54.4	54.8	57.3	60.2	62.4
2225	10.27.75	53.8	54.3	56.3	58.4	60.0	63.3	65.9
2227	11.14.75	53.4	54.0	55.4	57.1	58.2	59.8	61.4
2228	11.19.75	52.5	53.7	55.5	57.7	59.5	62.9	65.4
2229	11.20.75	49.9	50.6	52.2	53.9	54.7	57.7	61.0
2231	12.12.75	53.3	53.7	56.0	57.1	58.4	61.3	65.0
2232	12.16.75	51.9	52.4	54.6	55.8	56.6	59.9	62.4
2234	12.31.75	52.7	54.4	56.2	58.6	60.1	63.9	65.5
2236	1.13.76	51.4	52.6	53.5	54.8	56.9	58.7	62.3
2237	1.12.76	53.5	54.4	55.8	57.7	60.1	63.1	68.1
2239	2. 4.76	54.5	56.9	54.9	56.7	58.5	61.6	64.5

TABLE 2
Weights of individual infants fed formula I-501

Subject	Birth weight	8	14	28	42	56	84	112
				days				
Unsupplemented								
2221	3050	3065	3190	3552	4055	4535	5285	6025
2222	3145	3218	3355	4036	4661	5037	5743	6341
2223	3035	2997	3096	3525	4116	4771	5671	6322
2224	3375	3457	3566	3847	4057	4354	4949	5425
2226	3800	3721	3985	4730	5350	5849	6645	7365
2230	4000	4079	4199	4719	4914	5331	6101	6659
2233	3460	3489	3639	4488	5242	5798	6893	7542
2235	3345	3468	3624	4143	4560	5105	5853	6396
2238	3655	3510	3600	4058	4521	4803	5550	6207
2240	3260	3110	3360	3540				
Supplemented with methionine								
2220	3855	3669	3726	4064	4328	4949	5557	6174
2225	3800	4038	4230	4830	5335	5825	6595	7270
2227	4140	4004	3970	4237	4332	4700	5240	5300
2228	4000	3964	4075	4740	5176	5605	6070	6618
2229	3230	3431	3808	4422	4846	5343	6180	7015
2231	3740	3825	3919	4392	4876	5234	5631	6363
2232	3815	3750	4020	4661	5047	5531	6241	6861
2234	3970	4105	4325	5110	5805	6130	6885	7605
2236	3600	3662	4002	4815	5381	5673	6402	7200
2237	3925	3710	3953	4227	5072	5978	7500	8530
2239	4735	4730	4847	5485	5923	6564	7771	8664

TABLE 3
Average daily volume (ml) of formula consumed by individual infants

Subject	Age interval (days)					
	8–13	14–27	28–41	42–55	56–83	84–111
Unsupplemented						
2221	410	568	692	786	716	728
2222	565	734	766	761	731	688
2223	537	707	822	776	780	686
2224	429	499	550	569	642	615
2226	783	880	943	948	879	993
2230	570	655	805	892	928	950
2233	729	991	1116	1046	948	855
2235	657	806	835	877	883	886
2238	657	730	731	633	662	606
2240	493	585				
Supplemented with methionine						
2220	549	623	603	727	737	798
2225	747	816	782	843	863	839
2227	385	488	449	660	496	522
2228	689	801	698	719	742	845
2229	748	855	751	787	818	806
2231	659	663	737	769	798	889
2232	690	742	775	821	746	747
2234	740	823	920	886	856	866
2236	726	789	760	776	778	869
2237	571	506	877	1135	1072	1002
2239	801	907	886	951	966	923

TABLE 5
Average daily weight (g) of beikost consumed by individual infants (foods not permitted)

Subject	Age interval	Food designation	Quantity
	days		*g/day*
2224	56–83	Bananas	81
2226	56–83	Similac	35
2238	42–55	Pears	11
2239	56–83	Applesauce	13

TABLE 4
Average daily weight (g) of beikost consumed by individual infants[a]

Subject	Age interval (days)	Oatmeal with applesauce and bananas				Pears		Applesauce	Bananas
		28–41	42–55	65–83	84–111	56–83	84–111	84–111	84–111
Unsupplemented									
2221		4	31	44	43	5	4	8	4
2222		9	52	18	70	18	92	20	
2223		14	12	67	42	41	33	23	37
2224				27	44			65	
2226									
2230									
2233			73	55	69	26	8	22	18
2235					28				27
2238			52	158	136	5	14	4	110
Supplemented with methionine									
2220		44	32	49	68	52	21	25	61
2225		18	27	22	45		23		
2227				143	50	75	38	51	34
2228		60	68	65	55	24	9	13	7
2229		57	46	73	72	37	23	14	30
2231					22				13
2232			22	32	59	30	20	31	16
2234				23	30	7	9	9	18
2236		50		9	8	3	5	6	
2237		41	66	75	79	82	38	10	56
2239				110	82	40	27	32	13

[a]Missing values indicate that the infant did not consume the designated food in that age interval.

Requirement for Sulfur-Containing Amino Acids in Infancy

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ABSTRACT A series of studies designed to define the requirement of normal infants for sulfur-containing amino acids (methionine, cystine) was conducted with formulas providing 3.0, 2.8, 2.6, 2.2 or 1.8 g of isolated soy protein per 100 kcal. The formulas were fed with or without a methionine supplement. Adequacy of the diet was determined by measurement of growth, serum chemical indices and nitrogen balance. Nitrogen balance demonstrated a beneficial effect of methionine supplementation only at the lowest protein concentration (1.8 g/100 kcal). However, measurement of weight gain and/or serum concentrations of urea nitrogen and albumin clearly showed a beneficial effect of methionine supplementation at protein concentrations of 2.2 and 2.6 g/100 kcal. Intakes of sulfur-containing amino acids of 435 and 495 μmol/100 kcal therefore appear inadequate. At higher intakes of protein (2.8 and 3.0 g/100 kcal) there was no beneficial effect of methionine supplementation. Possible exceptions were male infants provided with 3.0 g protein per 100 kcal, in whom weight gain between 8 and 56 d of age was significantly ($P < 0.05$) greater with than without a methionine supplement. Based on intakes of sulfur-containing amino acids from the formula providing 2.8 g of isolated soy protein per 100 kcal without methionine supplementation, we conclude that for male infants older than 56 d the requirement for sulfur-containing amino acids is no more than 588 μmol/100 kcal when intake of methionine is 264 μmol/100 kcal. However, it seems possible that such intakes fail to meet the requirement in male infants less than 56 d of age. For female infants, regardless of age, 533 μmol/100 of sulfur-containing amino acids per 100 kcal meet the requirement when intake of methionine is 239 μmol/100 kcal. J. Nutr. *116*: 1405–1422, 1986.

INDEXING KEY WORDS sulfur-containing amino acids • methionine • isolated soy protein • normal infants

Requirements of infants for methionine were estimated by Snyderman et al. (1) with diets containing mixtures of 18 crystalline L-amino acids. Seven male infants, including three preterm infants, were studied. Total nitrogen intakes were approximately 480 mg/100 kcal, equivalent to a protein intake of 3.0 g/100 kcal. Cystine comprised 2.14% by weight of the amino acid mixture (2), resulting in cystine intake of approximately 530 μmol/100 kcal. It was concluded that requirements for methionine of individual infants ranged from 210 to 322 μmol/100 kcal. Under the circumstances of the studies, intake of total sulfur-containing amino acids was 740–852 μmol/100 kcal. For 4 of the 7 infants (2 full term infants under 2 mo of age and

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2 preterm infants over 2 mo of age) methionine intakes of 210–224 μmol/100 kcal (total sulfur-containing amino acid intakes of 740–754 μmol/100 kcal) were deemed inadequate.

The requirement for methionine estimated by Snyderman et al. is considerably greater than the intake by breast-fed infants. Mean protein intakes of breast-fed infants have been estimated to be 1.86 g/100 kcal during the first 2 mo of life, and 1.39 g/100 kcal between 2 and 4 mo of age (3). With concentration of methionine 114 μmol/g of human milk protein (4), intakes of methionine average 212 μmol/100 kcal during the first 2 mo of life and 159 μmol/100 kcal between 2 and 4 mo of age. The cystine concentration of human milk is 223 μmol/g protein (4) (i.e., 415 μmol/100 kcal during the first 2 mo of life and 310 μmol/100 kcal from 2 to 4 mo of age), and the concentration of taurine is about 45 μmol/100 kcal (5). Thus, the intake of total sulfur-containing amino acids of breast-fed infants is estimated to be 672 μmol/100 kcal in the first 2 mo of life and 513 μmol/100 kcal between 2 and 4 mo of age.

The conclusion of Snyderman et al. (1) that intakes of sulfur-containing amino acids of 740–754 μmol/100 kcal failed to meet the requirements of several infants may be a result of the study design. Evaluation of performance was based on nitrogen balance and weight gain. However, in most instances in which methionine intakes of 224 μmol/100 kcal or less were fed, nitrogen balance appeared satisfactory and the conclusion regarding inadequacy of methionine intake was based solely on weight gain over brief intervals (generally 5–13 d). For such intervals, the distinction between adequate and inadequate growth of an individual infant is difficult. Moreover, in some instances, infants were fed diets devoid of methionine, followed by a diet providing 151 μmol of methione per 100 kcal before being fed the diet providing 210–224 μmol of methionine per 100 kcal. Thus, some methionine depletion may have preceded the evaluation of the diet providing 740 to 754 μmol of sulfur-containing amino acids per 100 kcal.

Because of the uncertainties of the Snyderman et al. (1) conclusion about the requirement for methionine, we decided to conduct further studies. Isolated soy proteins are attractive for studies of requirements for sulfur-containing amino acids because concentrations of methionine and cystine per unit of protein are low in relation to concentrations of non-sulfur-containing essential amino acids. In 1979 we (6) reported a study of two groups of infants fed a formula in which protein was supplied in the form of isolated soy protein unfortified with methionine. The formula provided 2.24 g protein (354 mg of nitrogen) per 100 kcal, an amount well above the requirement when cow milk-based formulas are fed and, as already noted, greater than amounts consumed by breast-fed infants. One group of infants was given a supplement of L-methionine in drop form with each feeding. The findings were inconclusive. Although there was no significant difference in rates of growth of infants in the two feeding groups, mean serum concentration of urea nitrogen was significantly less in the supplemented infants. Bodwell (7) has summarized evidence indicating that when proteins of different quality are fed at similar intakes of nitrogen, animals consuming the protein of lesser quality demonstrate greater serum concentrations of urea nitrogen and our results therefore suggested that the addition of L-methionine improved protein quality. In addition, although not statistically significant, nitrogen retention was slightly greater when infants were given the L-methionine supplement. We now report additional studies in which infant formulas prepared from isolated soy protein with no added methionine were fed with or without a methionine supplement. The adequacy of the diet was judged by observations of food intake, growth, serum chemical indices and nitrogen balance. We believe that the results provide better estimates of the infant's requirement for sulfur-containing amino acids than do those previously available.

SUBJECTS

Normal term infants with birth weights of 2500 g or more (appendix 1) were studied. The study protocols were reviewed

and approved by the University of Iowa Committee on Research Involving Human Subjects. At the time an infant was enrolled in the study, at least one of the parents was interviewed by B. B. Edwards. The program was described in detail, written permission was obtained and written instructions were provided. Two types of study were conducted: *1*) "growth studies," consisting of determination of food intake, growth and serum chemical values, and 2) metabolic balance studies.

Infants serving as subjects for the growth studies were enrolled between 6 and 9 d of age, and the majority completed the planned 112 d of observation. Until the time of enrollment, most of these infants had been fed commercially prepared milk-based formulas. Infants serving as subjects for the metabolic balance studies described here had generally served previously as subjects for other metabolic balance studies. These infants did not participate in the growth studies.

FEEDINGS

The experimental formulas were prepared for us by two manufacturers: Wyeth Laboratories, Radnor, PA, prepared formula 2.6; the other formulas were prepared by Ross Laboratories, Columbus, OH. Except for differences in protein concentration and omission of the usual supplement of L-methionine, the formulas were similar to commercially available isolated soy protein formulas. The formulas were supplied to us ready to feed, approximately 67 kcal/dL, in 240-mL glass bottles to which a nipple assembly could be attached. Each formula was prepared by the manufacturer as a single batch. Protein was provided from Edipro A (Ralston Purina Co., St. Louis, MO), an isolated soy protein that provides 87 μmol of methionine and 107 μmol of cystine per gram of protein (8). We have designated the formulas in relation to the approximate protein concentration (g/100 kcal) as indicated in table 1: formulas 3.0, 2.8, 2.6, 2.2 and 1.8. Fat was provided as a mixture of vegetable oils: formulas 3.0, 2.8, 2.2 and 1.8 contained 60% soy oil and 40% corn oil; formula 2.6 contained 33% oleo oil, 27% coconut oil, 25% high oleic safflower oil and 15% soy oil. Carbohydrate consisted of 70% corn syrup solids and 30% sucrose in formulas 3.0, 2.8, 2.2 and 1.8, and 100% sucrose in formula 2.6. Further information regarding the composition of the formulas is presented in table 1.

A supply of formula sufficient for 7 d was weighed and delivered to the family. When a new supply was delivered 7 d later, the bottles from the previous supply, including any unconsumed amounts of formula, were collected and again weighed. Bottles for each 24-h period were weighed separately. In addition to the formula, each infant received a daily supplement of 0.25 mg of fluoride (Karidium Liquid, Lorvic Corporation, St. Louis, Missouri). When the experimental design called for the addition of methionine, one dropperful of the methionine solution, approximately 82 μmol of methionine, was added to each 240 mL (163 kcal) of formula (i.e., 50 μmol/100 kcal) shortly before feeding. The L-methionine was dissolved in distilled water, 82 μmol/mL, and was provided in 120-mL glass bottles with a dropper that delivered approximately 1 mL.

In contrast to feeding practices in the earlier study (6), the parents were instructed not to feed beikost ("solid" foods) and, except for minor infractions that are noted in appendix 2, the formula served as sole source of food.

PROCEDURES

Growth studies

Infants were studied from the time of enrollment (between 6 and 9 d of age) to 112 d of age. Body weight and length were determined as described previously (9). Measurements were made between 6 and 9 d of age, within 2 d of ages 14, 28, 42 and 56 d and within 4 d of ages 84 and 112 d. A detailed description of the intervals of study and the way in which they are applied to data analysis has been presented (10). The methods for obtaining venous blood and for analyses of serum, foods and excreta were the same as in previous studies (10). Serum concentrations of urea nitrogen and albumin were performed as described previously (11).

TABLE 1

Composition of feedings[1]

	Formula				
	3.0	2.8	2.6	2.2	1.8
Major constituents, *g/100 kcal*[2]					
Protein[3]	3.03	2.75	2.55	2.24	1.75
Fat[4,5]	5.44	5.29	5.27	5.41	5.29
Carbohydrate[4,6]	10.00	10.00	10.69	10.59	10.59
Minerals, *per L*					
Calcium, *mg*	1163	1071	961	947	706
Phosphorus, *mg*	829	734	628	599	540
Magnesium, *mg*	75	85	110	75	85
Sodium, *meq*	19.3	24.0	13.9	21.3	17.5
Chloride, *meq*	22.8	20.3	18.1	22.8	32.9
Potassium, *meq*	32.5	21.5	26.7	24.7	35.6
Iron, *mg*	20.1	18.2	24.6	23.7	29.0
Zinc, *mg*	8.5	7.8	8.8	8.8	7.1
Copper, *mg*	1.09	1.35	0.70	1.87	1.01
Manganese, *mg*[7]	0.74	0.44	0.84	–	0.54

[1]Formulas 3.0, 2.8, 2.2 and 1.8 were supplemented as follows (weight/liter): iodine, 100 μg; vitamin A, 2000 IU; vitamin D, 400 IU; vitamin E, 20 IU; vitamin K, 0.1 mg; vitamin C, 55 mg; thiamin, 0.40 mg; riboflavin, 0.60 mg; vitamin B-6, 0.40 mg; vitamin B-12, 3.0 μg; niacin, 9 mg; folic acid, 100 μg; pantothenic acid, 5 mg; biotin, 30 μg; choline, 53 mg; inositol, 32 mg. Formula 2.8 was supplemented as follows: iodine, 65 μg; vitamin A, 2500 IU; vitamin D, 400 IU; vitamin E, 9 IU; vitamin K, 0.1 mg; vitamin C, 55 mg; thiamin, 0.67 mg; riboflavin, 1 mg; vitamin B-6, 0.4 mg; vitamin B-12, 2 μg; niacin, 9.5 mg; folic acid, 50 μg; pantothenic acid, 3 mg; biotin, 35 μg; choline, 85 mg; inositol, 26 mg. [2]Calculated metabolizable energy of each formula was 67 or 68 kcal/dL. [3]Edipro A. [4]Manufacturer's analysis. Other values are those determined by authors. [5]60% soy oil and 40% corn oil except for formula 2.6, where fat was 33% oleo oil, 27% coconut oil, 25% high oleic safflower oil and 15% soy oil. [6]Sucrose in formula 2.6; in all other formulas 70% corn syrup solids and 30% sucrose. [7] Manganese concentration of formula 2.2 was not determined.

Two different study designs were used, referred to subsequently as longitudinal and balanced crossover. In studies using a longitudinal design (those with formulas 3.0 and 2.2) the same formula with or without the methionine supplement was fed from the time of enrollment through 112 d of age. In studies using a balanced crossover design (those with formulas 2.8 and 2.6), each infant was fed the unsupplemented formula at some times and the supplemented formula at other times. Subjects in group A received no supplement from 8–27 d and from 56–83 d of age; they received the supplement from 28–55 d and from 84–111 d of age. Subjects in group B were given the supplement in the opposite sequence, beginning with the supplement in the interval 8–27 d of age.

Formula 3.0. Male and female infants were studied using a longitudinal design. Seventeen male infants were enrolled, nine in the group that did not receive the methionine supplement (group 3.0) and eight in the group that received the supplement (group 3.0m). One infant in group 3.0 was withdrawn from the study soon after the visit at 14 d of age because the family was away from Iowa City for several weeks. Data on this infant are included in appendices 1 and 2. Nineteen female infants were enrolled. Initially, nine were entered into group 3.0 and eight into group 3.0m. Twins were then enrolled and, through an error, both rather than one were assigned to group 3.0. All 19 infants completed the planned period of observation.

Formula 2.8. Female infants were studied using a balanced crossover design. Eighteen infants were enrolled and 16 completed the planned period of observation, 8 in group A and 8 in group B. One infant was withdrawn from the growth study at 22 d of age to be enrolled in metabolic balance studies, and one infant was withdrawn from the study at 42 d of age because of constipation attributed to the

formula. Data on the two infants who failed to complete the study are included in appendices 1 and 2.

Formula 2.6. Male infants were studied with a balanced crossover design. Eighteen male infants were enrolled, and 15 completed the planned period of observation, 7 in group A and 8 in group B. One infant was withdrawn from the study after the visit at 84 d of age because the family moved from the city. Two other infants were withdrawn from the study after the visit at 14 d of age, one because of fussiness and spitting up and one because of blood in the stools. Data on these infants are included in appendices 1 and 2.

Formula 2.2. Male infants were studied using a longitudinal design. As reported earlier (6), 19 male infants completed the study from 8 to 112 d of age while consuming an isolated soy protein formula that provided 2.2 g protein/100 kcal (designated in this report as formula 2.2).

Reference data. To aid the reader in evaluating the performance of infants fed the various levels of isolated soy protein with and without methionine supplementation, data from infants fed cow milk-based formulas are included. The data concern 81 male and 63 female infants studied with a longitudinal design. Dates of birth of these infants spanned the period from September 1974 through March 1984. Dates of birth of infants in the methionine supplementation studies, including the previously reported one (6), ranged from June 1975 through January 1984. The milk-based formulas were either commercially available or similar to commercially available milk-based formulas. Protein concentrations ranged from 2.09 to 2.69 g/100 kcal (1.4. to 1.8 g/dL), and hence intakes of sulfur-containing amino acids (12) ranged from 533 to 686 μmol/100 kcal. Data on energy intake and growth of these infants are presented in table 2.

Metabolic balance studies

Metabolic balance studies were carried out with each of the five formulas in a balanced crossover design whereby each infant received the formula without supplement during some balance studies and with supplement during other balance studies. The formula under study, with or without the supplement, was fed for at least 11 d before beginning a balance study. Balance studies of 72 h duration were performed as described previously (9) with three infants fed formula 3.0, four infants fed formula 2.8, three infants fed formula 2.6, four infants fed formula 2.2 and five infants fed formula 1.8. The number of infants studied was determined to a major extent by the number available to us at the time.

Data analysis

The analyses of data pertain only to infants who completed the planned period of observation from 8 to 112 d of age. Data on length, weight and formula intake of the other infants are included in appendices 1 and 2.

For growth studies with longitudinal design, one-way analysis of variance was used to compare energy intakes and growth for the various age intervals. For the studies with a crossover design (those with formulas 2.8 and 2.6), the age intervals 8–27, 28–55, 56–83 and 84–111 d were analyzed separately. Linear contrasts (13) were used to compare performance during periods with supplementation with that during periods without supplementation and for comparison with performance of infants fed milk-based formulas. Serum concentrations of urea nitrogen and albumin were analyzed by a repeated-measures analysis of covariance. Data from the metabolic balance studies were analyzed by a randomized block, mixed effects model. All statistical analyses were performed by the general linear models procedure of the SAS statistics program (SAS Institute, Inc. Cary, NC).

RESULTS

Growth studies

It will be helpful to the reader to know that performance (i.e., growth, serum chemical values and/or nitrogen balance) of infants fed formulas 3.0 and 2.8 without supplemental methionine was considered adequate, with the possible exception of male infants less than 56 d of age. On the other hand, performance of infants

TABLE 2

Reference data: energy intake and gains in length and weight of infants fed cow milk-based formulas

Measure	Mean ± SD	Centiles 10	25	50	75	90
A. Males (n=81)						
Energy intake, *kcal/kg per d*						
8– 27 d	119 ± 16	99	109	119	128	139
28– 55 d	113 ± 13	95	105	114	121	129
56– 83 d	99 ± 9	89	92	99	104	113
84–111 d	92 ± 8	82	86	92	100	103
8– 55 d	116 ± 13	98	107	117	124	132
8–111 d	105 ± 8	96	99	104	111	117
Length gain, *mm/d*						
8– 28 d	1.4 ± 0.3	1.1	1.2	1.4	1.6	1.9
28– 56 d	1.2 ± 0.2	0.9	1.0	1.2	1.4	1.5
56– 84 d	1.0 ± 0.2	0.7	0.9	1.1	1.2	1.3
84–112 d	0.9 ± 0.2	0.6	0.7	0.9	1.0	1.2
8– 56 d	1.3 ± 0.2	1.1	1.1	1.3	1.4	1.5
8–112 d	1.1 ± 0.1	1.0	1.0	1.1	1.2	1.2
Weight gain, *g/d*						
8– 28 d	43 ± 9	30	37	43	49	55
28– 56 d	37 ± 8	27	31	36	41	48
56– 84 d	28 ± 7	19	24	28	33	36
84–112 d	22 ± 6	13	17	22	27	30
8– 56 d	39 ± 7	29	34	40	44	48
8–112 d	32 ± 5	25	28	32	35	39
Weight gain, *g/100 kcal*						
8– 28 d	9.0 ± 1.5	6.8	8.1	8.9	10.0	10.9
28– 56 d	6.5 ± 1.1	5.0	5.9	6.6	7.2	7.8
56– 84 d	4.8 ± 1.0	3.5	4.2	4.8	5.4	5.9
84–112 d	3.6 ± 0.9	2.4	2.9	3.6	4.1	4.7
8– 56 d	7.4 ± 1.0	6.0	7.0	7.5	8.0	8.8
8–112 d	5.6 ± 0.7	4.8	5.2	5.6	6.1	6.6
B. Females (n=63)						
Energy intake, *kcal/kg per d*						
8– 27 d	120 ± 12	106	110	118	130	138
28– 55 d	115 ± 10	102	106	115	122	130
56– 83 d	104 ± 8	92	99	105	110	114
84–111 d	97 ± 9	87	93	95	102	107
8– 55 d	117 ± 10	103	109	117	124	133
8–111 d	108 ± 7	99	102	109	114	117
Length gain, *mm/d*						
8– 28 d	1.4 ± 0.2	1.1	1.2	1.4	1.5	1.7
28– 56 d	1.1 ± 0.2	0.8	0.9	1.1	1.2	1.4
56– 84 d	1.0 ± 0.2	0.7	0.8	0.9	1.1	1.2
84–112 d	0.9 ± 0.2	0.6	0.8	0.9	1.1	1.2
8– 56 d	1.2 ± 0.1	1.0	1.1	1.2	1.3	1.4
8–112 d	1.1 ± 0.1	0.9	1.0	1.0	1.1	1.2
Weight gain, *g/d*						
8– 28 d	35 ± 8	24	29	35	41	45
28– 56 d	31 ± 8	21	25	30	35	42
56– 84 d	26 ± 6	18	22	25	30	33
84–112 d	22 ± 6	13	19	22	26	31
8– 56 d	32 ± 7	24	27	33	37	42
8–112 d	28 ± 5	23	25	28	31	35
Weight gain, *g/100 kcal*						
8– 28 d	7.7 ± 1.3	5.9	6.6	7.7	8.6	9.5
28– 56 d	5.7 ± 1.0	4.3	5.0	5.7	6.5	7.1
56– 84 d	4.6 ± 0.9	3.4	4.0	4.6	5.2	5.8
84–112 d	3.8 ± 1.0	2.5	3.3	3.7	4.5	4.9
8– 56 d	6.5 ± 0.9	5.4	5.9	6.4	7.1	7.7
8–112 d	5.2 ± 0.7	4.4	4.7	5.2	5.6	6.1

fed formulas 2.6 and 2.2 was considered adequate only with supplemental methionine. Data on length, weight and formula intake of individual infants are presented in appendices 1 and 2, except for previously reported data (6) for infants fed formula 2.2.

Formula 3.0. Data on energy intake, gain in length and gain in weight by infants fed formula 3.0 are presented in table 3. Among male infants, energy intake and gain in length in groups 3.0 and 3.0m during all age intervals were similar to those of male infants fed milk-based formulas (table 2A). For the age interval 8–112 d, gain in weight (g/d) by group 3.0 was somewhat lower than that by group 3.0m or by the milk-based formula group. However, the differences were not statistically significant ($P = 0.051$ and 0.324, respectively). Similarly, gain in weight (g/100 kcal) was not significantly different in groups 3.0, 3.0m and the milk-based formula group. However, for the age interval 8–55 d gain in weight (g/d) of male infants was significantly less by group 3.0 than by group 3.0m ($P = 0.025$) or by the milk-based formula group ($P = 0.043$), whereas energy intake and gain in length were not significantly different by infants in group 3.0, group 3.0m and the milk-based formula group. Gains in weight per unit of energy

TABLE 3

Food intake and growth by infants fed formula 3.0[1]

Measure	Males		Females	
	3.0 (8)	3.0m[2] (8)	3.0 (11)	3.0m (8)
Size at age 8 d[3]				
Length, *mm*	509 ± 22	511 ± 12	504 ± 18	513 ± 12
Weight, *g*	3517 ± 420	3570 ± 413	3379 ± 359	3593 ± 230
Energy intake, *kcal/kg per d*				
8– 27 d	116 ± 12	125 ± 16	124 ± 7	111 ± 15
28– 55 d	111 ± 11	116 ± 11	115 ± 7	111 ± 9
56– 83 d	102 ± 8	100 ± 14	98 ± 7	96 ± 12
84–111 d	93 ± 7	90 ± 8	91 ± 7	91 ± 7
8– 55 d	113 ± 10	120 ± 12	118 ± 6	111 ± 8
8–111 d	105 ± 5	107 ± 8	106 ± 6	102† ± 6
Length gain, *mm/d*				
8– 28 d	1.27 ± 0.22	1.23 ± 0.40	1.28 ± 0.36	1.26 ± 0.30
28– 56 d	1.19 ± 0.21	1.27 ± 0.28	1.26 ± 0.17	1.20 ± 0.16
56– 84 d	0.97 ± 0.21	1.22 ± 0.21	0.94 ± 0.21	0.99 ± 0.22
84–112 d	0.95 ± 0.18	0.78 ± 0.30	0.83 ± 0.19	0.89 ± 0.31
8– 56 d	1.23 ± 0.13	1.25 ± 0.16	1.27 ± 0.21	1.23 ± 0.12
8–112 d	1.08 ± 0.09	1.12 ± 0.13	1.06 ± 0.11	1.07 ± 0.15
Weight gain, *g/d*				
8– 28 d	37.3 ± 11.6	45.4 ± 9.5	38.7 ± 9.6	37.2 ± 9.2
28– 56 d	31.2 ± 8.1	40.0 ± 10.9	34.1 ± 6.3	34.0 ± 4.2
56– 84 d	28.7 ± 6.5	33.4 ± 13.0	26.9 ± 5.5	24.1 ± 9.4
84–112 d	24.1 ± 4.5	24.6 ± 10.1	20.0 ± 6.0	22.2 ± 9.0
8– 56 d	33.8[a,*] ± 7.1	42.3 ± 9.5	36.0 ± 5.4	35.3 ± 5.0
8–112 d	29.8 ± 2.2	35.1 ± 9.3	29.3 ± 4.6	28.8 ± 4.2
Weight gain, *g/100 kcal*				
8– 28 d	8.14 ± 1.72	8.99 ± 1.11	8.35 ± 1.92	8.43 ± 1.42
28– 56 d	5.97 ± 1.48	6.74 ± 1.12	6.37 ± 0.84	6.31 ± 0.49
56– 84 d	5.07 ± 1.04	5.26 ± 1.47	4.98 ± 0.78	4.33 ± 1.46
84–112 d	4.11 ± 0.66	3.84 ± 1.15	3.61 ± 1.09	3.81 ± 1.36
8– 56 d	6.83 ± 1.02	7.61 ± 1.04	7.14* ± 0.87	7.08 ± 0.61
8–112 d	5.56 ± 0.39	5.90 ± 1.02	5.54 ± 0.76	5.42 ± 0.50

[1]Superscripts indicate statistical significances, with letters ([a], $P < 0.05$) referring to comparisons between supplemented and unsupplemented formulas within same sex and same age interval, and symbols (*, $P < 0.05$; †$P < 0.01$) referring to comparisons with reference data (table 2) within same sex and same age interval; (*n*), number of subjects. [2]An m indicates that a supplement of methionine was given. [3]Values are means ± SD.

intake by the three groups were not significantly different.

In the case of females (table 3), there were no significant differences in comparisons of the three groups of infants except that from 8–111 d of age energy intake was less by group 3.0m than by the milk-based formula group ($P = 0.006$) and that from 8–55 d of age gain in weight per unit of energy intake was greater by group 3.0 than by the milk-based formula group ($P = 0.044$). We have observed a significant difference in energy intake between feeding groups in a number of our studies and do not have a satisfactory explanation, especially when it occurs as an isolated difference between groups. It seems unsound to conclude that inadequate composition of the diet was responsible for greater food consumption because the difference in energy intake between feeding groups was at times in the opposite direction (see results with formulas 2.6 and 2.6m).

Data on serum concentrations of urea nitrogen and albumin of infants fed formula 3.0 are summarized (sexes combined) in table 4. No effect of methionine supplementation on serum concentration of urea nitrogen was noted (analysis of covariance with age as covariate). Because serum concentration of urea nitrogen is influenced by protein intake (7) and because the range of protein intakes was lower from milk-based than from soy-based formulas, values for urea nitrogen of infants fed the former have not been included. Similar analysis failed to reveal statistically significant differences in serum concentration of albumin between group 3.0, group 3.0m and the milk-based formula group.

Formula 2.8. Data on energy intake and growth by females fed formula 2.8 are presented in table 5. Energy intake, gain in length and gain in weight were similar for infants in a specified age interval whether they were being fed formula 2.8, formula

TABLE 4

Serum concentrations of urea nitrogen and albumin in relation to methionine (Met) supplementation[1]

Measure	Formula 3.0	Formula 2.8	Formula 2.6	Formula 2.2	Milk-based
Urea nitrogen, *mg/dl*[2]					
Without Met					
28 d	(14) 14.2 ± 2.8	(4) 15.6 ± 2.2	(5) 11.8 ± 0.5	(8) 11.5[a] ± 1.3	
56 d	(16) 13.4 ± 2.9	(6) 13.8 ± 1.3	(7) 11.3 ± 1.4	(8) 10.2[a] ± 1.3	
84 d	(16) 12.5 ± 2.0	(8) 13.2 ± 2.2	(6) 10.1 ± 2.2	(7) 10.1[a] ± 1.7	
112 d	(15) 12.6 ± 1.5	(6) 11.3 ± 0.4	(7) 11.6 ± 2.5	(9) 9.4[a] ± 1.6	
With Met					
28 d	(10) 15.1 ± 3.0	(5) 14.6 ± 2.1	(7) 11.2 ± 2.1	(8) 9.5 ± 1.7	
56 d	(13) 13.9 ± 2.4	(5) 14.3 ± 2.8	(5) 10.7 ± 1.6	(8) 8.6 ± 1.4	
84 d	(14) 13.9 ± 2.1	(6) 12.2 ± 2.7	(7) 11.3 ± 1.6	(9) 8.1 ± 1.3	
112 d	(14) 12.9 ± 2.0	(5) 12.6 ± 1.4	(5) 9.7 ± 0.8	(8) 7.5 ± 1.6	
Albumin, *g/dl*					
Without Met					
28 d	(14) 3.72 ± 0.27	(4) 3.68 ± 0.18	(5) 3.53[a,*] ± 0.22	(8) 3.40[‡] ± 0.32	(129) 3.80 ± 0.28
56 d	(16) 4.07 ± 0.27	(6) 3.99 ± 0.17	(7) 4.01 ± 0.42	(8) 3.53[a,‡] ± 0.47	(124) 4.00 ± 0.28
84 d	(17) 4.18 ± 0.38	(8) 4.07 ± 0.18	(6) 3.90[b,†] ± 0.21	(7) 3.98* ± 0.22	(118) 4.16 ± 0.24
112 d	(15) 4.23 ± 0.29	(6) 4.16 ± 0.18	(7) 4.29 ± 0.19	(9) 4.03* ± 0.26	(122) 4.25 ± 0.26
With Met					
28 d	(10) 3.94 ± 0.24	(5) 3.90 ± 0.12	(7) 3.94 ± 0.23	(8) 3.56* ± 0.25	
56 d	(13) 4.01 ± 0.16	(5) 4.01 ± 0.33	(5) 3.92 ± 0.08	(8) 3.88 ± 0.25	
84 d	(14) 4.23 ± 0.17	(6) 4.20 ± 0.22	(7) 4.27 ± 0.15	(9) 3.92[†] ± 0.24	
112 d	(14) 4.30 ± 0.32	(5) 4.13 ± 0.34	(5) 4.03 ± 0.33	(8) 4.07* ± 0.20	

[1]Superscripts indicate statistical significances, with letters ([a] $P < 0.05$; [b] $P < 0.01$) referring to comparisons between supplemented and unsupplemented formulas within same age interval, and symbols (* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$) referring to comparisons with reference data within same age interval. [2]Values are means ± SD; (*n*), number of subjects.

TABLE 5

Food intake and growth by infants fed formulas 2.8 and 2.6 in studies using a crossover design[1]

Measure	Formula 2.8 (females)		Formula 2.6 (males)	
	Group A (8)	Group B (8)	Group A (7)	Group B (8)
Size at age 8 d[2]				
Length, *mm*	496 ± 13	504 ± 11	511 ± 15	508 ± 20
Weight, *g*	3160 ± 393	3542 ± 373	3397 ± 407	3449 ± 315
Energy intake, *kcal/kg per d*				
8– 27 d	122 ± 17	116[m] ± 10	127 ± 25	123[m] ± 9
28– 55 d	109[m] ± 10	112 ± 14	132[m,‡] ± 22	123 ± 15
56– 83 d	106 ± 12	99[m] ± 7	114‡ ± 21	111[m,†] ± 10
84–111 d	91[m] ± 5	102[a] ± 10	111[m,‡] ± 18	99[a] ± 10
Length gain, *mm/d*				
8– 28 d	1.21 ± 0.41	1.43[m] ± 0.18	1.20[a,*] ± 0.40	1.51[m] ± 0.28
28– 56 d	1.16[m] ± 0.30	1.03 ± 0.26	1.04[m] ± 0.21	1.00 ± 0.17
56– 84 d	0.99 ± 0.19	0.94[m] ± 0.13	1.02 ± 0.16	1.14[m] ± 0.08
84–112 d	0.95[m] ± 0.16	0.96 ± 0.20	0.87[m] ± 0.30	1.01 ± 0.21
Weight gain, *g/d*				
8– 28 d	32.3 ± 5.8	32.8[m] ± 8.4	27.0[a,‡] ± 12.2	37.4[m] ± 10.8
28– 56 d	30.1[m] ± 8.5	27.7 ± 6.5	33.0[m] ± 8.9	31.2 ± 8.7
56– 84 d	22.2 ± 8.5	23.0[m] ± 5.5	27.6 ± 10.1	30.6[m] ± 7.1
84–112 d	22.4[m] ± 5.5	21.5 ± 7.1	27.7[m,*] ± 11.5	24.5 ± 8.3
Weight gain, *g/100 kcal*				
8– 28 d	7.79 ± 0.94	7.37[m] ± 1.88	5.79[a,‡] ± 2.29	7.89[m] ± 1.79
28– 56 d	5.93[m] ± 1.23	5.89 ± 1.13	5.76[m] ± 1.49	5.50* ± 1.48
56– 84 d	4.22 ± 1.38	4.27[m] ± 0.70	4.57 ± 1.67	4.97[m] ± 0.89
84–112 d	3.74[m] ± 0.97	4.04 ± 0.68	4.25[m] ± 1.73	3.95 ± 1.10

[1]Superscripts indicate statistical significances, with letters (*, $P < 0.05$) referring to comparisons between supplemented and unsupplemented formulas within same sex and same age interval, and symbols (*$P <$ 0.05; †$P < 0.01$; ‡$P < 0.001$) referring to comparisons with reference data (table 2) within same sex and same age interval. [m]Period in which L-methionine supplement was given. [2]Values are means ± SD; (*n*), number of subjects.

2.8m (table 5) or the milk-based formulas (table 2*B*). An exception concerned energy intake from 84–111 d of age. In this interval infants of group A, who were receiving the supplement, demonstrated less energy intake than did those of group B (not supplemented) ($P = 0.029$) or those fed milk-based formulas (not significant, $P = 0.195$). As already mentioned, we do not consider this difference between groups A and B to suggest inadequacy of formula 2.8 without methionine.

Serum concentrations of urea nitrogen and albumin (table 4) were similar during periods in which the supplement was given and periods in which no supplement was given. Changes in serum albumin from 28 to 56, 56 to 84 and 84 to 112 d failed to demonstrate an effect of methionine supplementation.

Formula 2.6. In each age interval, energy intake by infants fed formula 2.6 or formula 2.6m (table 5) was greater than that by infants fed the milk-based formulas (table 2*A*) and for several of the intervals the difference was significant. For the entire interval 8–111 d of age, energy intake by group A was 120 kcal/kg per d and that by group B was 114 kcal/kg per d. These intakes are greater than the 105 kcal/kg per d of the milk-based formula group ($P < 0.001$, $P = 0.022$, respectively). The greater intakes by infants fed formula 2.6 may be related to the carbohydrate used (all sucrose). We have demonstrated that under circumstances of ad libitum feeding infants consume greater amounts of a formula in which all of the carbohydrate is sucrose than of an otherwise similar formula in which all of the carbohydrate is a corn starch hydrolysate of minimal sweetness (14). In the age interval 84–111 d energy intake was significantly greater ($P = 0.012$) by infants fed formula 2.6m than

by infants fed formula 2.6. As already noted, there seems to be no reason to conclude that methionine supplementation would result in increased food consumption, and we are inclined to ignore the finding.

Gain in length from 8 to 28 d of age was less by infants fed formula 2.6 than by infants fed formula 2.6m ($P = 0.042$) or by infants fed the milk-based formulas ($P = 0.035$). In each of the other age intervals, gains in length were similar by infants fed formula 2.6, formula 2.6m and by the group fed milk-based formula.

Gain in weight in each age interval tended to be less by infants fed formula 2.6 than by those fed formula 2.6m, but the difference was statistically significant only for the age interval 8–28 d ($P = 0.034$). In this age interval mean gain in weight by the unsupplemented infants was also less than that by the milk-based formula group ($P < 0.001$).

Gain in weight per unit of energy intake in each age interval tended to be less by infants fed formula 2.6 than by those fed formula 2.6m but, as in the case of gain expressed as grams per day, the difference was statistically significant only for the age interval 8–28 d ($P = 0.012$). Both in this age interval and in the age interval 28–56 d gain in weight per unit of energy intake (g/100 kcal) was less by infants fed formula 2.6 than by those fed the milk-based formulas ($P < 0.001$, $P = 0.018$, respectively).

Serum concentrations of urea nitrogen and albumin are presented in table 4. No effect of methionine supplementation on serum concentration of urea nitrogen was noted. Serum concentrations of albumin at ages 28 and 84 d were significantly less for the infants fed formula 2.6 than for those fed formula 2.6m or for those fed milk-based formulas (table 4). Although not indicated in table 4, within each group (groups A and B) the change in serum concentration of albumin in the successive age intervals, 28–56, 56–84 and 84–112 d in each instance tended to be greater during periods of supplementation than during periods without supplementation. Although the difference was statistically significant for the age interval 28–56 d ($P = 0.041$), it was not significant for the age intervals 56–84 d ($P = 0.051$), or 84–112 d ($P = 0.801$).

Based on the lower gain in length and weight and on the difference in serum albumin concentration, the performance of male infants fed formula 2.6 without methionine supplementation was considered inadequate.

Formula 2.2. A summary of data on energy intake and growth of male infants fed formula 2.2 is presented in table 6. For the age interval 8–111 d, energy intake was greater by group 2.2 than by group 2.2m (not significant, $P = 0.057$) or by the milk-based formula group ($P = 0.006$). Gain in length was similar for groups 2.2 and 2.2m and the milk-based formula group. Differences in gain in weight (g/d and g/100 kcal) between groups 2.2 and 2.2m and the milk-based formula group were not statistically significant.

In the age interval 8–55 d, energy intake by group 2.2 was not significantly different from that by group 2.2m or by the milk-based formula group. Gain in weight by group 2.2 tended to be slightly less than that by group 2.2m ($P = 0.284$) but was significantly ($P = 0.030$) less than that by the milk-based formula group. Similarly, gain in weight per unit of energy intake tended to be less by group 2.2 than by group 2.2m (not significant, $P = 0.281$) and was significantly ($P = 0.003$) less than that by the milk-based formula group.

Data on serum concentrations of urea nitrogen and albumin are summarized in table 4. Concentration of urea nitrogen was significantly greater in group 2.2 than in group 2.2m ($P = 0.009$). Analysis of covariance with age as the covariate failed to reveal a significant difference in serum concentration of albumin between groups 2.2 and 2.2m. Concentration of albumin in group 2.2 was significantly less than in the milk-based formula group ($P = 0.001$). Concentration of albumin was not significantly less in group 2.2m than in the milk-based formula group ($P = 0.058$).

Although we initially were uncertain about the interpretation of our results from studies of formulas 2.2 and 2.2m (6), reanalysis of the data with the additional reference group of infants fed milk-based formulas now leads us to conclude that

TABLE 6

Food intake and growth by males fed formula 2.2[1]

Measure	Formula 2.2 (9)	Formula 2.2m (10)
Size at age 8 d[2]		
Length, *mm*	506 ± 13	525 ± 13
Weight, *g*	3445 ± 332	3888 ± 357
Energy intake, *kcal/kg per d*		
8– 27 d	124 ± 24	116 ± 14
28– 55 d	122 ± 15	110 ± 12
56– 83 d	108 ± 6	102 ± 11
84–111 d	99 ± 5	98 ± 9
8– 55 d	123 ± 17	113 ± 6
8–111 d	113[b] ± 8	106 ± 6
Length gain, *mm/d*		
8– 27 d	1.40 ± 0.24	1.20 ± 0.41
28– 55 d	1.10 ± 0.27	1.17 ± 0.28
56– 83 d	1.00 ± 0.18	1.09 ± 0.19
84–111 d	1.02 ± 0.14	1.06 ± 0.34
8– 55 d	1.22 ± 0.21	1.18 ± 0.23
8–111 d	1.11 ± 0.07	1.12 ± 0.41
Weight gain, *g/d*		
8– 27 d	33.9 ± 11.1	39.3 ± 12.0
28– 55 d	33.7 ± 9.8	36.0 ± 9.7
56– 83 d	28.2 ± 5.0	28.6 ± 12.0
84–111 d	22.2 ± 3.1	26.7 ± 5.2
8– 55 d	33.7[a] ± 9.3	37.4 ± 6.1
8–111 d	29.1 ± 5.9	32.1 ± 6.8
Weight gain, *g/100 kcal*		
8– 27 d	7.19 ± 1.19	7.80 ± 1.77
28– 55 d	5.96 ± 1.23	6.24 ± 0.89
56– 83 d	4.77 ± 0.47	4.45 ± 1.14
84–111 d	3.65 ± 0.57	3.99 ± 0.56
8– 55 d	6.39[b] ± 0.92	6.88 ± 0.77
8–111 d	5.14 ± 0.63	5.35 ± 0.70

[1]Superscripts indicate statistical significances; [a]$P < 0.05$; [b]$P < 0.01$ referring to comparisons with reference data (table 2) within same sex and same age interval. [2]Values are means ± SD; (*n*), number of subjects.

the addition of methionine increased the nutritional quality of the formula.

Metabolic balance studies

Results of the balance studies are summarized in table 7, and data from individual balance studies, except for those with formula 2.2 [previously reported (6)], are given in appendix 3.

When infants are fed the same formula under similar conditions, as in consecutively performed metabolic balance studies in our unit, intakes of nitrogen are generally quite similar. This similarity in intakes, which was observed in the paired balance studies with and without the L-methionine supplement, permits the use of nitrogen retention as an indication of the advantage of supplementation. With formulas 3.0, 2.8, 2.6 and 2.2 no statistically significant effect of methionine supplementation on retention of nitrogen was demonstrated. However, at the lowest nitrogen concentration (formula 1.8) an effect of methionine supplementation on retention of nitrogen was observed. This effect was statistically significant ($P = 0.001$). Individual values for studies with formula 1.8 are presented in figure 1.

DISCUSSION

Some years ago we attempted to estimate the requirements of infants for sulfur-containing amino acids on the basis of growth and nitrogen balance of infants fed formulas with cow milk protein or with methionine-supplemented isolated soy protein (15). Data from other investigators (16–22) and from our laboratory (23) concerning growth of infants fed soy formulas can also be used for estimating requirements for sulfur-containing amino acids, although in most instances the intakes of formula were not precisely measured. We believe that the estimates we made in 1973 are less reliable than those now presented from studies of infants fed isolated soy protein formulas with and without a supplement of L-methionine. A similar approach has been used in studies of adult subjects (24–26). Interpretation of data from the studies presented here is facilitated by the inclusion of a reference group of infants observed concurrently while being fed cow milk-based formulas.

In the metabolic balance studies reported here, a beneficial effect of methionine supplementation on nitrogen balance was demonstrated only when isolated soy protein was fed at a low level of protein intake—1.75 g/100 kcal (formula 1.8)—providing 340 μmol of sulfur-containing amino acids per 100 kcal. Similar findings have been reported by Graham (27) in studies of children convalescing from malnutrition. Eight children 6–23 mo of age were fed soy flour or isolated soy

TABLE 7

Effect of methionine supplementation on nitrogen balance of infants less than 210 d of age

Feeding[1]	No. Subjects	No. Studies	Nitrogen balance			
			Intake	Excretion		Retention
				Urine	Feces	
			mg/kg per d			
3.0	3	6	563 (125)[2]	240 (33)	93 (32)	230 (72)
3.0m	3	6	551 (92)	248 (31)	104 (28)	199 (69)
2.8	4	8	592 (34)	268 (49)	99 (20)	225 (53)
2.8m	4	8	586 (43)	287 (43)	101 (23)	197 (39)
2.6	3	5	444 (83)	227 (21)	96 (15)	121 (55)
2.6m	3	5	467 (77)	212 (23)	100 (27)	155 (46)
2.2	4	7	374 (76)	181 (30)	73 (16)	120 (52)
2.2m	4	7	380 (51)	173 (31)	70 (17)	137 (41)
1.8	5	10	318 (39)	136 (14)	59 (15)	122* (22)
1.8m	5	10	318 (52)	117 (16)	52 (14)	149* (47)

[1]An m indicates that the supplement of L-methionine was given. [2]Values in parentheses are standard deviations. [2]Difference in retention with and without the supplement is statistically significant ($P < 0.01$).

protein with or without a supplement of methionine. The studies were carried out at low protein intakes: 1.0 g/100 kcal in three children and 1.25 to 1.75 g/100 kcal in the others. In each instance a beneficial effect of methionine supplementation was demonstrated. Assuming that soy flour provides 94 μmol of methionine and 132 μmol of cystine per gram of protein and that isolated soy protein provides 87 μmol of methionine and 107 μmol of cystine per gram of protein (8) the unsupplemented diets of the eight children provided 87 to 165 μmol of methionine and 107 to 231 μmol of cystine g/100 kcal. Thus we conclude that intakes of methionine of 87 to 165 μmol/100 kcal with intake of total sulfur-containing amino acids of 194 to 396 μmol/100 kcal were inadequate for young children during convalescence from malnutrition.

From our studies and those of Graham (27), we conclude that a beneficial effect of methionine supplementation on nitrogen balance can be demonstrated when the intake of sulfur-containing amino acids is 396 μmol/100 kcal or less. However, with greater intakes of sulfur-containing amino acids (e.g., 435 to 495 μmol/100 kcal, as with formulas 2.2 and 2.6), nitrogen balance appears to be an insensitive index of protein quality.

Contrary to nitrogen balances, gain in weight and/or serum concentrations of urea nitrogen and albumin demonstrated a beneficial effect of methionine supplementation with intakes of sulfur-containing amino acids of 435 μmol/100 kcal (formula 2.2) and 495 μmol/100 kcal (formula 2.6). Such intakes therefore appear to be inadequate.

No evidence of a beneficial effect of methionine supplementation was evident in female infants fed isolated soy protein at a level of 2.8 or 3.0 g/100 kcal. The indices evaluated were gain in length, gain in weight, serum concentrations of urea nitrogen and albumin, and nitrogen balance studies. From these observations we conclude that the intakes of isolated soy protein were adequate to meet the requirements of females for protein and for essential amino acids. An intake of 2.75 g of protein per 100 kcal resulted in an intake of 239 μmol of methionine and 294 μmol of cystine per 100 kcal. We conclude that for female infants, 533 μmol of total sulfur-containing amino acids per 100 kcal meet the requirement when intake of methionine is 239 μmol/100 kcal.

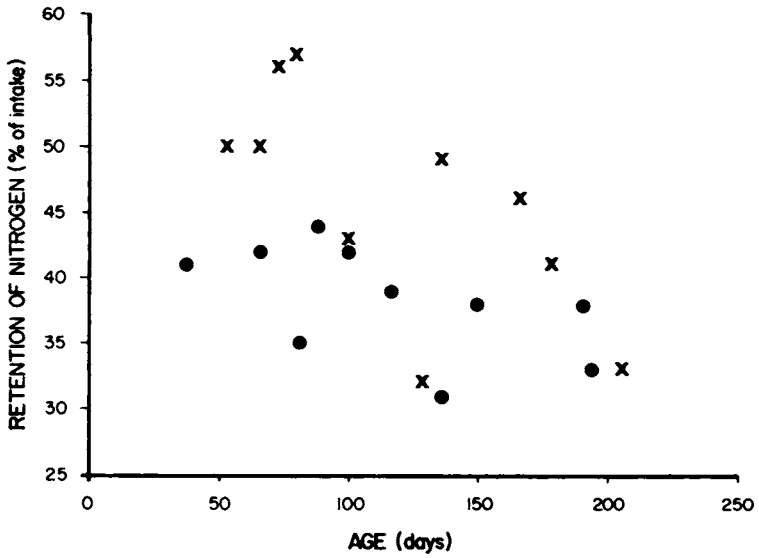

Fig. 1 Retention of nitrogen expressed as percent of intake in relation to age of infants fed formula 1.8 without (●) or with (×) a supplement of L-methionine.

The requirement for sulfur-containing amino acids of male infants is not so clearly identified by our studies. In the age interval 8–56 d, male infants fed formula 3.0 without a supplement of methionine gained weight less rapidly ($P = 0.025$) than infants receiving the same feeding with a supplement of methionine and less rapidly ($P = 0.043$) than a group of male reference infants fed milk-based formulas. None of the other indices of performance was significantly different beteen male infants fed the isolated soy protein with or without the methionine supplement or those fed the milk-based formulas. Weight gain by the male infants fed the unsupplemented formula in the age interval 56–112 d was well within anticipated limits, being slightly (not significantly) greater than that by the group of male reference infants (table 2A) fed milk-based formulas.

We conclude that for male infants older than 56 d the requirement for sulfur-containing amino acids is no more than that supplied by the isolated soy protein fed at 3.0 g/100 kcal; i.e., 588 μmol/100 kcal when 264 μmol/100 kcal are provided by methionine. However, it seems possible that such an intake fails to meet the requirement of male infants during the age interval 8–56 d.

As already indicated, during the first 2 mo of life our estimate of intake of methionine from human milk is 212 μmol/100 kcal and that of total sulfur-containing amino acids (including taurine) is 672 μmol/100 kcal. With these intakes, weight gain of breast-fed males is 37 g/d in the age interval 8–56 d (11)—only slightly less than the 39 g/d of males fed milk-based formulas (table 2A). Males in our study who were fed the isolated soy protein at 3.0 g/100 kcal without the methionine supplement received 588 μmol/100 kcal of total sulfur-containing amino acids and gained 33.8 g/d. On the other hand, with the methionine supplement intake of total sulfur-containing amino acids in the age interval 8–55 d of age was 638 μmol/100 kcal and weight gain was 42.3 g/d.

The possibility that a sex-related difference might exist in requirement for sulfur-containing amino acids for infants 8–56 d of age is not totally implausible. As may be seen from table 2, gain in weight was substantially more rapid by males than by females during the early weeks of life. From

TABLE 8

Methionine (Met) intake and weight gain by male infants receiving various formulas; age 8–56 d

	Weight gain[1]				
	Milk-based	SCAA[2]	Formula 2.2	SCAA[2]	Formula 3.0
	g/d		*g/d*		*g/d*
Without added Met	39 ± 7 (81)	435	33.7 ± 9.3 (9)	588	33.8 ± 7.1 (8)
With added Met	–	485	37.4 ± 6.1 (10)	638	42.3 ± 9.3 (8)

[1]Values are means ± SD; (*n*), number of subjects. [2]Intake of sulfur-containing amino acids (SCAA) expressed as μmol/100 kcal.

8 to 56 d of age mean gain by male infants fed milk-based formulas was 39 g/d (table 2*A*). The corresponding gain by female infants was 32 g/d (table 2*B*). Assuming that at this age 11.2% of the gain is protein (28), protein accretion may be estimated at 4.37 g/d for males and 3.58 g/d for females. This difference was not eliminated by expressing protein accretion per unit of body weight or per unit of energy intake. With a greater rate of protein accretion by males than by females, one would anticipate greater requirements for protein and essential amino acids. After 56 d of age, the difference in rate of protein accretion by male and female infants is much less than in the younger infant. For example, estimated protein accretion from 56 to 112 d of age was 2.80 g/d for males and 2.58 g/d for females.

Nevertheless, we are not convinced that formula 3.0 unsupplemented with methionine was inadequate for male infants in the age interval 8–56 d. There was no difference in length gain. The relatively low weight gain may have been a chance occurrence, a possibility that is difficult to ignore in view of the small number of infants (8) in the feeding group. In support of this interpretation are comparisons presented in table 8 concerning weight gains by male infants fed milk-based formulas and formulas 2.2 and 3.0. For formulas 2.2 and 3.0 the intakes of sulfur-containing amino acids per unit of energy intake are given without the methionine supplement and with the supplement of 50 μmol/100 kcal. When formula 3.0 was fed without the methionine supplement, the intake of sulfur-containing amino acids was 588 μmol/100 kcal and weight gain in the age interval 8–56 d was, as previously discussed, significantly less than that by infants fed the same formula supplemented with methionine or by infants fed milk-based formulas. If this relatively low rate of weight gain were the result of inadequate intake of sulfur-containing amino acids, one would anticipate that a further decrease in intake of sulfur-containing amino acids per unit of energy intake would result in more dramatic effects on weight gain. Actually, with an intake of sulfur-containing amino acids of 485 μmol/100 kcal by infants fed formula 2.2 with the methionine supplement, weight gain was not significantly different from that of infants fed milk-based formulas. We therefore consider it unlikely that the slow rate of gain of infants fed formula 3.0 without the methionine supplement was a consequence of inadequate intake of sulfur-containing amino acids.

Because rate of body protein accretion decreases with increasing age (28), requirements for sulfur-containing amino acids per unit of energy intake will be less for older than for younger infants. We conclude that throughout infancy the requirement for sulfur-containing amino acids is no more than 588 μmol/100 kcal under circumstances in which 264 μmol/100 kcal is methionine (as supplied by formula 3.0 unsupplemented with methionine). However, this conclusion is somewhat less well supported for males during the first 56 d of life than for older males or for females.

APPENDIX 1

Lengths and weights of infants

Subject no.	Birth date	Length, mm							Weight, g							
		Age, d							Age, d							
		8	14	28	42	56	84	112	Birth	8	14	28	42	56	84	112
Formula 3.0 Males																
2661	04/29/80	507	520	533	545	561	589	613	3700	3540	3680	4115	4641	5111	6184	6789
2663	05/09/80	502	511	–	–	–	–	–	3570	3590	3925	–	–	–	–	–
2665	06/27/80	507	519	526	551	569	598	625	3035	3356	3660	4285	4905	5280	5940	6550
2667	08/01/80	540	542	570	582	600	617	638	3855	3816	4030	4915	5285	5790	6253	7100
2671	09/29/80	494	501	526	536	560	591	622	3515	3548	3690	4245	4905	5130	6000	6673
2673	11/22/80	513	520	540	555	572	598	629	3345	3352	3533	4060	4667	5040	5956	6535
2675	12/16/80	485	489	510	536	551	573	595	3090	3101	3202	3986	4658	5050	5820	6338
2676	12/19/80	485	496	506	526	539	576	598	3005	3065	3180	3405	3690	4265	5085	5950
2678	01/29/81	541	557	564	579	591	619	654	4280	4360	4574	5096	5233	5435	6287	6979
Formula 3.0m Males																
2662	05/06/80	499	501	517	542	552	596	623	3090	3153	3483	4341	5129	5761	6959	8062
2664	05/16/80	507	518	534	551	563	602	607	3570	3526	3725	4365	4880	5220	5775	6130
2666	07/18/80	539	544	572	592	614	650	671	3915	4319	4560	5345	5885	6470	7365	8025
2668	09/02/80	509	514	537	541	560	585	604	3970	3911	4061	4568	4950	5420	6459	7440
2670	09/17/80	506	516	540	554	571	600	632	3685	3635	3914	4767	5399	6131	7095	7785
2672	11/15/80	503	510	524	539	562	594	623	3260	3118	3437	3952	4165	4896	5950	6473
2674	11/27/80	516	525	527	564	574	605	622	3740	3678	3924	4405	4829	5212	5522	5852
2677	01/25/81	509	511	534	563	573	611	635	3315	3220	3468	4078	5082	5680	7153	8021
Formula 3.0 Females																
2641	12/01/79	496	509	517	541	554	583	604	2975	2943	3175	3775	4291	4636	5414	5971
2643	12/15/79	506	515	532	549	568	603	618	3035	3189	3454	4066	4457	4829	5752	6341
2645	01/04/80	521	529	549	558	582	609	636	3770	3895	4170	4925	5547	5935	6850	7515
2647	02/11/80	517	521	529	540	564	590	618	3230	3289	3425	3870	4135	4720	5365	5935
2649	02/24/80	518	525	537	566	574	598	625	3515	3565	3810	4321	5053	5497	6093	6736
2651	03/07/80	536	548	556	568	586	608	623	3940	4067	4230	4522	5212	5681	6281	6370
2653	04/01/80	490	500	516	529	542	577	598	3800	3610	3795	4265	4775	5100	5765	6284
2655	05/28/80	493	508	522	537	553	576	603	3120	3229	3390	3895	4555	5060	5935	6680
2658	07/27/80	479	491	514	530	553	567	598	3090	3080	3254	3737	4321	4396	4911	5460
2659	08/02/80	495	511	530	552	572	603	626	3090	3213	3611	4230	4738	5297	6131	6711
2660	08/02/80	487	503	519	539	560	586	610	2890	3087	3270	4084	4503	5053	5994	6638
Formula 3.0m Females																
2642	12/03/79	516	525	552	565	585	618	652	3485	3575	3860	4245	4760	5125	5715	6455
2644	12/17/79	509	524	537	558	572	599	620	3400	3342	3560	4260	4825	5195	5900	6400
2646	02/08/80	492	496	511	527	541	558	579	3290	3286	3461	3792	4176	4573	5403	5610
2648	02/16/80	524	536	552	567	586	611	638	4110	3968	4082	4756	5249	5687	5819	6720
2650	03/05/80	522	524	544	560	578	607	622	3740	3665	3950	4495	4955	5345	6000	6380
2652	03/23/80	525	531	541	565	584	605	627	3770	3781	3917	4448	5097	5546	6228	6776
2654	04/03/80	517	526	542	560	571	606	646	3515	3684	3857	4223	5051	5292	6356	7190
2656	06/07/80	502	509	529	543	559	593	612	3415	3444	3774	4482	5038	5553	6288	7140
Formula 2.8 Females																
2911	11/15/81	514	514	521	539	551	571	598	3970	3841	4009	4337	4665	5031	5199	5629
2913	11/29/81	482	491	506	530	549	575	602	2520	2635	2769	3219	3635	3925	4428	4613
2915	12/16/81	495	502	523	540	562	597	618	3090	3131	3370	4000	4585	5110	5880	6610
2917	01/07/82	477	489	511	527	545	571	594	3005	3040	3261	3660	4254	4670	5575	6373
2919	01/24/82	508	516	537	551	562	593	623	3630	3390	3596	4053	4360	4688	5180	5824
2921	02/07/82	510	513	–	–	–	–	–	3770	3910	3945	–	–	–	–	–
2923	02/14/82	487	495	509	537	550	575	598	2835	2670	2754	3238	3638	3986	4642	5287
2925	03/25/82	495	502	518	525	538	562	598	3175	3289	3481	4035	4318	4663	5527	6247
2927	03/29/82	507	514	534	552	561	596	622	3315	3285	3516	3905	4311	4585	5209	5876
Formula 2.8m Females																
2912	11/19/81	489	498	524	534	–	–	–	3740	3846	4056	4597	4949	–	–	–
2914	12/12/81	495	498	530	540	561	588	607	3460	3557	3701	4224	4807	5213	5987	6694
2916	12/22/81	482	491	504	523	533	564	591	3190	2992	3175	3667	4063	4432	4767	5376
2918	01/25/82	509	514	539	548	564	593	624	3715	3550	3761	4243	4640	4947	5534	6093
2920	01/28/82	515	531	545	568	582	611	640	3770	3735	3793	4213	4825	5330	6146	6950
2922	02/17/82	513	521	540	553	564	588	610	3940	3896	4065	4400	4730	5165	5835	6720
2924	03/22/82	510	515	535	543	557	582	603	4110	4097	4230	4560	4860	5100	5640	6124
2926	03/26/82	500	510	532	540	554	573	610	3345	3393	3730	4250	4455	4905	5627	6113
2928	04/03/82	507	520	533	556	574	601	630	3175	3119	3312	4030	4714	5244	5949	6444
Formula 2.6 Males																
3067	04/07/83	484	494	509	527	545	570	599	2805	2710	2887	3425	4086	4440	5520	6379
3071	07/07/83	503	511	524	539	553	579	591	2950	3297	3358	3651	4087	4419	4692	5371
3075	07/26/83	498	501	520	537	545	584	–	2905	3015	3005	3462	3893	4358	5556	–
3076	08/09/83	515	526	545	557	562	598	627	3515	3357	3537	4080	4503	4849	5673	6216
3078	08/29/83	523	525	545	563	575	605	631	3655	3744	3937	4455	5020	5505	6080	6987
3079	09/10/83	503	508	523	533	553	577	589	3120	3103	3106	3406	3845	4253	4993	5246
3081	10/13/83	505	512	–	–	–	–	–	3360	3355	3420	–	–	–	–	–
3083	11/04/83	525	528	537	553	568	595	621	3830	3736	3705	3975	4329	4630	5500	6470
3085	11/27/83	525	544	557	570	587	619	655	3940	3830	4042	4618	5078	6033	7072	8303
Formula 2.6m Males																
3066	03/26/83	530	542	564	575	594	623	662	3485	3736	4041	4508	4937	4999	5892	6814
3068	04/16/83	520	529	542	559	574	608	635	3500	3589	3572	4035	4459	5256	5993	6800
3070	07/04/83	494	502	520	531	541	570	592	3545	3427	3595	4030	4315	4615	5575	6175
3072	07/17/83	487	498	520	532	551	581	602	3485	3134	3325	4113	4620	5102	5940	6254
3074	08/07/83	517	524	546	559	571	604	629	3460	3250	3423	3943	4336	4972	5771	6812
3077	08/27/83	530	543	557	567	580	612	641	4240	3986	4138	5135	5423	5887	7108	7706
3080	09/13/83	477	481	–	–	–	–	–	2975	3010	2940	–	–	–	–	–
3082	10/20/83	512	524	542	562	577	612	645	3430	3425	3562	4097	4616	5111	6022	6681
3084	11/07/83	477	502	522	535	550	582	613	4365	3046	3325	3815	4255	4765	5320	6042

APPENDIX 2

Average daily volume (mL) of formula consumed by individual infants[1]

Subject	Age interval, d					
	8–13	14–27	28–41	42–55	56–83	84–111
Formula 3.0 Males						
2661	479	620	786	757	923	862
2663	622	–	–	–	–	–
2665	711	784	860	819	816	881
2667	670	834	875	859	860	938
2671	480	707	837	819	883	820
2673	516	659	763	688	893	891
2675	564	761	854	844	803	749
2676	465	563	547	766	775	860
2678	698	782	636	723	795	969
Formula 3.0m Males						
2662	735	884	971	979	1016	1033
2664	700	863	892	812	635	777
2666	817	920	935	964	972	1024
2668	556	661	691	875	1014	1092
2670	680	904	921	1014	932	890
2672	544	679	646	868	947	899
2674	574	721	739	704	693	683
2677	589	723	926	1058	1072	1010
Formula 3.0 Females						
2641	530	680	700	677	754	765
2643	628	750	755	895	904	922
2645	782	891	850	879	866	838
2647	637	678	666	705	779	820
2649	650	736	892	908	823	798
2651	775	764	914	914	863	911
2653	551	657	731	697	719	793
2655	523	680	772	869	829	850
2658	588	687	768	697	625	744
2659	492	778	806	840	848	845
2660	480	769	797	805	831	869
Formula 3.0m Females						
2642	699	668	757	772	788	864
2644	459	778	923	874	835	869
2646	642	613	672	710	772	763
2648	587	745	781	718	587	845
2650	656	729	758	804	800	722
2652	500	729	847	867	862	790
2654	523	428	912	775	940	985
2656	643	790	863	831	847	950

Subject	Age interval, d					
	8–13	14–27	28–41	42–55	56–83	84–111
Formula 2.8 Females						
2911	468	640	660	725	654	786
2913	446	544	512	460	605	609
2915	654	773	869	896	862	890
2917	492	608	690	769	838	939
2919	520	638	655	751	770	876
2921	597	–	–	–	–	–
2923	589	684	676	740	833	904
2925	575	733	781	777	827	846
2927	646	688	639	672	783	855
Formula 2.8m Females						
2912	488	527	448	–	–	–
2914	500	671	795	773	859	905
2916	565	614	633	640	604	697
2918	620	703	753	708	774	787
2920	691	641	789	859	880	928
2922	759	712	752	777	913	939
2924	670	640	686	680	729	787
2926	730	749	651	767	758	775
2928	506	742	906	905	841	780
Formula 2.5 Males						
3067	557	614	761	735	770	753
3071	424	502	584	609	607	797
3075	569	785	1001	1063	1086	–
3076	675	947	971	1023	1002	1016
3078	628	684	846	826	891	1137
3079	573	630	853	980	1054	1090
3081	–	–	–	–	–	–
3083	599	675	928	971	950	990
3085	853	1051	1003	1134	985	1151
Formula 2.6m Males						
3066	716	777	788	846	984	1012
3068	516	763	947	1041	991	967
3070	544	616	627	737	819	893
3072	577	775	856	885	921	813
3074	593	785	881	923	980	1087
3077	780	897	1020	1111	1074	956
3080	583	–	–	–	–	–
3082	601	688	778	847	862	919
3084	558	648	686	679	696	774

[1]Additional food intake was as follows: subject 2653, 60 g strained oatmeal, age 42–55 d; subject 2648, 570 g Pedialyte, 56–83 d; subject 2650, 1358 g Seven-Up, 84–111 d; subject 2664, 1260 g skim milk and 30 g sucrose (in water), 56–83 d; subject 2913, Karo syrup 15 g 14–27 d, 75 g 28–41 d, 360 g 42–55 d, 420 g 56–83 d, and Pedialyte 4313 g 84–111 d; subject 3078, 750 g Similac with iron, 14–27 d.

Appendix G

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APPENDIX 3

Nitrogen balances in relation to methionine supplementation

Subj.	Sex	Age	Wt	Lt	Energy intake	Balance*			
						I	U	F	R
		d	*g*	*cm*	*kcal / (kg · d)*	*mg/(kg · d)*			
Formula 3.0									
2498	M	20	4120	52.9	157	752	272	152	328
		48	5490	56.5	138	662	272	111	280
2497	F	135	7680	61.8	93	436	222	74	141
		163	7770	64.0	100	452	186	77	188
2496	F	141	6105	60.7	105	504	250	75	179
		169	6820	63.3	119	572	239	70	263
Formula 3.0m									
2498	M	34	5010	54.5	119	571	224	101	246
		62	5885	58.3	123	590	239	157	194
2496	F	113	5660	58.4	138	663	291	106	266
		155	6485	61.8	126	603	253	94	256
2497	F	121	7465	60.5	90	419	208	75	136
		149	7500	63.5	89	462	273	89	100
Formula 2.8									
2921	F	22	4250	52.4	157	667	279	131	256
		50	4845	54.6	138	587	272	124	190
2936	M	37	4190	50.9	134	571	265	72	234
		65	4955	53.8	135	574	290	99	185
2500	F	128	5140	57.8	133	565	265	96	204
		156	5610	59.6	136	572	284	97	191
2499	F	188	5690	61.2	139	587	157	87	343
		215	6120	62.9	147	612	328	83	200
Formula 2.8m									
2921	F	36	4700	53.8	149	634	268	142	223
		64	5705	56.2	133	568	299	116	153
2936	M	51	4765	52.2	129	550	240	102	208
		79	5345	54.6	130	555	249	78	229
2500	F	114	4940	56.8	127	539	249	116	175
		142	5340	58.8	158	662	313	88	261
2499	F	159	5435	60.3	137	583	318	96	169
		201	5940	61.9	141	594	362	72	159
Formula 2.6									
2939	F	157	6420	65.8	95	371	196	84	91
2940	M	188	5605	59.8	85	337	215	82	40
		265	6055	63.7	133	494	237	120	137
2941	M	241	9120	71.2	143	506	244	91	170
		276	9605	72.4	144	511	245	101	166
Formula 2.6m									
2939	F	143	6435	64.7	93	359	188	75	96
2940	M	174	5380	58.5	107	421	195	87	139
		251	5980	62.1	142	549	207	121	220
2941	M	227	8690	68.2	141	518	240	135	144
		262	9250	71.2	137	490	233	82	175
Formula 1.8									
2482	M	37	4560	55.6	122	335	136	62	138
		79	6140	60.0	112	311	133	71	108
2481	M	66	4730	56.6	120	319	140	44	134
		101	5250	59.4	99	289	114	54	122
2299	F	87	5110	58.4	112	295	124	42	128
		116	5450	60.0	130	380	161	69	150
2298	F	136	5530	59.3	109	297	152	53	92
		192	6240	62.5	93	249	124	43	82
2300	M	149	7500	65.1	119	327	129	73	125
		191	7965	67.7	141	373	147	84	142
Formula 1.8m									
2481	M	52	4400	54.9	142	378	138	50	190
		80	4965	57.6	118	320	107	32	181
2482	M	65	5650	58.4	132	360	107	71	181
		128	7435	64.6	96	241	97	67	78
2299	F	73	4815	56.9	117	378	97	71	210
		101	5160	59.1	109	300	125	45	130
2300	M	135	7105	64.0	135	371	142	48	181
		177	7825	67.4	125	303	121	58	124
2298	F	164	5865	60.9	106	284	108	44	132
		206	6500	63.2	89	245	126	38	80

*I, intake; U, urinary excretion; F, fecal excretion; R, retention.

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COMMITTEE REPORT

Comment on the Composition of Soy Protein Based Infant and Follow-up Formulas

ESPGAN COMMITTEE ON NUTRITION: P. J. AGGETT (Secretary), F. HASCHKE, W. HEINE, O. HERNELL, K. LAUNIALA, J. REY (Chairman), A. RUBINO, G. SCHÖCH, J. SENTERRE and R. TORMO

The ESPGAN Committee on Nutrition has published recommendations for the composition of adapted formulas (1) and follow-up formulas based on cow's milk (2, 3). This report considers the composition of infant and follow-up formulas based on soy isolate proteins. The clinical indications for soy isolate protein products are debatable. Indications for which they are often used are: (a) adverse reactions to cow's milk protein, (b) a requirement for lactose and/or galactose free diets, and (c) an alternative for those who wish to avoid giving their infants formulas containing animal products (4). Although the second and third indications justify the choice of a soy-based formula, the Committee considers that available data do not support the view that such formulas should be the preferred choice when suspected, or proven adverse effects to cow's milk protein is the indication (5, 6). Certainly the availability of soy-based products should not compromise the important concept, that an infant's own mother's milk is the most appropriate feed.

ENERGY

Soy based infant formulas

250–315 $kJ \cdot dl^{-1}$ (60–75 $kcal \cdot dl^{-1}$)

Soy based follow-up formulas

250–335 $kJ \cdot dl^{-1}$ (60–80 $kcal \cdot dl^{-1}$)

The metabolisable energy content of infant feeding formulas based on soy-protein isolates is similar to that of formulas based on cow's milk. The Committee can therefore consider that the energy density of these formulas can correspond to recommendations which have already been made for infant formulas and follow-up formulas based on cow's milk protein (1, 3).

PROTEIN

Soy based infant formulas

0.56–0.7 $g \cdot 100\ kJ^{-1}$
2.25–3.0 $g \cdot 100\ kcal^{-1}$
1.35–2.25 $g \cdot dl^{-1}$

NB.: Minimum methionine content of 7.3 mg (50 μmol)·100 kJ^{-1}, i.e. 30 mg (200 μmol)·100 $kcal^{-1}$. Minimum L-carnitine content of 0.3 mg (1.8 μmol)·100 kJ^{-1}, i.e. 1.2 mg (7.5 μmol)·100 $kcal^{-1}$.

Soy based follow-up formulas

0.7–1.1 g·100 kJ^{-1}
3.0–4.5 g·100 $kcal^{-1}$
1.8–3.6 g·dl^{-1}

NB.: Minimum methionine content of 7.3 mg (50 μmol)·100 kJ^{-1}, i.e. 30 mg (200 μmol)·100 $kcal^{-1}$. L-carnitine content of 0.3 mg (1.8 μmol)·100 kJ^{-1}, i.e. 1.2 mg (7.5 μmol)·100 $kcal^{-1}$.

Unmodified soy based milks are considered unsuitable for infants because of side effects caused by raffinose and stachyose (7). Isolated soy protein if appropriately processed is a good vegetable protein source for children (7). It has a high nutritional value and its amino acid composition rating is 96% that of casein, and even after allowance has been made for digestibility, the amino acid score is 89% overall and still remains above 80% when the least available amino acid, methionine, is considered, but nevertheless this is limiting (8). Thus even when protein intake is not marginal methionine supplements are needed to ensure growth, and to maintain nitrogen balance and circulating plasma albumin concentrations (9). The Committee considers therefore, that soy protein isolate based infant and follow-up formulas should contain at least 30 mg (200 μmol) of methionine·100 $kcal^{-1}$ (50 μmol (7.3 mg)·100 kJ^{-1}), approximating to the amount in human breast milk.

In contrast to human breast milk and formulas based on cow's milk protein, soy based products contain no intrinsic L-carnitine (10), the function of which is to transfer fatty acids into the mitochondria. The newborn infant has a finite store of carnitine which, in the absence of an exogenous supply, could be depleted by two and a half months (11–14). Therefore, although there is, with one possible exception (15), no conclusive evidence that infants fed soy based products are at serious risk of developing carnitine deficiency (11), the Committee consider it prudent to support the view that soy based products should be supplemented to a level approximating that in human breast milk.

FAT

Soy based infant formulas and soy based follow-up formulas

0.9–1.4 g·100 kJ^{-1}

4.0–6.0 g·100 $kcal^{-1}$

Since soy protein isolates are lipid-free, fat needs to be added. This is done by manufacturers using varying proportions of vegetable oils such as sunflower, safflower, coconut, palm, corn (maize) and occasionally oleo oils, thereby offering considerable opportunity to manipulate the lipid composition of the products. The Committee, at present, is not aware of any metabolic indications for using vegetable fats to the complete exclusion of animal fats though they appreciate that in some circumstances this may be preferred on cultural grounds. We see no reason at

present to have different recommendations on the lipid content from those recommended for infant formulas and follow-up formulas based on cow's milk protein (3). The lipid composition of infant formulas will be reviewed by the Committee in the future.

CARBOHYDRATE

Soy based infant formulas and soy based follow-up formulas

2.0–3.0 g·100 kJ^{-1}
8.0–12.0 g·100 $kcal^{-1}$

The absence of lactose from soy protein isolates has enabled the use of alternative carbohydrate sources and, thereby, the therapeutic use of soy based products in the management of children who need to avoid either lactose or galactose or both. Additionally since the facilitative effects of lactose on mineral absorption could be achieved also by glucose polymers the Committee discourages the specific supplementation of soy based products with lactose. If however lactose is present in such formulas the Committee recommends that the products should be labelled as "lactose containing".

Starch is sometimes added to soy based infant formulas, in which case it should be gluten free and starch should not exceed 3 g·100 $kcal^{-1}$. The addition of sucrose should be discouraged, but the Committee agrees that the amount of sucrose and, in the case of follow-up formulas, fructose and honey added separately or as a whole should not exceed 20% of the total carbohydrate content (16).

MINERALS

Calcium and phosphorus
Soy based infant formulas

Calcium: minimum	14 mg·100 kJ^{-1}
	60 mg·100 $kcal^{-1}$
	40 mg·dl^{-1}
Phosphorus:	7.2–12 mg·100 kJ^{-1}
	30–50 mg·100 $kcal^{-1}$
	20–35 mg·dl^{-1}

Ca: P ratio: not less than 1.2 and not more than 2.0.

Soy based follow-up formulas

Calcium: minimum	22 mg·100 kJ^{-1}
	90 mg·100 $kcal^{-1}$
	60 mg·dl^{-1}
Phosphorus:	14 mg·100 kJ^{-1}
minimum	60 mg·100 $kcal^{-1}$
	40 mg·dl^{-1}

Ca: P ratio: not less than 1.0 and not more than 2.0.

Soy based products, which have not been designed specifically for infants, are poor in calcium but rich in phosphorus, and infants fed these products have developed overt rickets (17). Poorer mineralisation of bone has also been observed

in infants receiving a soy protein isolate based infant formula, when compared with those fed infant formulas based on cow's milk protein (18, 19). However, these differences which were present at 3 months of age had disappeared at 6 months of age, and infants followed up until 1 year of age had bone mineralisation similar to those of breast fed and vitamin D supplemented infants (18, 19). Therefore the Committee recommends that the calcium and phosphorus content of soy based infant formulas and follow-up formulas should be similar to those for cow's milk based formulas (1, 3).

Iron and zinc

Iron 0.24–0.48 mg (4.3–8.6 μmol)·100 kJ^{-1} i.e. 1.0–2.0 mg (18.0–36 μmol)·100 $kcal^{-1}$.

Zinc Minimum 0.18 mg (2.8 μmol)·100 kJ^{-1}, i.e. 0.75 mg (11.5 μmol)·100 $kcal^{-1}$, with a maximum iron : zinc molar ratio of 2.5 : 1.

Native soy protein has a high (1–1.5%) content of phytate (inositol hexaphosphate) which is a potent chelator and inhibitor of the absorption of trace elements such as iron (20–23) and zinc (24–27). Evidently the ideal solution to the limited availability of iron and zinc from soy based formulas would be to remove all their phytate content. In the absence of achieving this the Committee feels that there is a need to enrich these products with both iron and zinc.

Interactions which limit intestinal uptake and transfer of some trace metals also occur between inorganic elements, thus iron may interfere with the utilisation of zinc and vice versa (28). Hence, it is important to consider the relative proportions of these metals in infant formulas and follow-up formulas. Although, in the future, it may be necessary to comment on the amount of copper, for the moment we make a recommendation only for iron and zinc in that we feel that the iron : zinc molar ratio should not exceed 2.5. The Committee proposes that, in contrast to the provision for cow's milk based formulas, soya protein isolate based products should be enriched with iron at 1.0–2.0 mg (18.0–36 μmol)·100 $kcal^{-1}$.

VITAMINS

The Committee considered that there was no reason to deviate from the Codex recommendations on vitamins which have been provided for cow's milk based infant formulas and follow-up formulas.

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Medical Position Paper

Soy Protein Infant Formulae and Follow-On Formulae: A Commentary by the ESPGHAN Committee on Nutrition

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ABSTRACT: This comment by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition summarizes available information on the composition and use of soy protein formulae as substitutes for breastfeeding and cows' milk protein formulae as well as on their suitability and safety for supporting adequate growth and development in infants. Soy is a source of protein that is inferior to cows' milk, with a lower digestibility and bioavailability as well as a lower methionine content. For soy protein infant formulae, only protein isolates can be used, and minimum protein content required in the current European Union legislation is higher than that of cows' milk protein infant formulae (2.25 g/100 kcal vs. 1.8 g/100 kcal). Soy protein formulae can be used for feeding term infants, but they have no nutritional advantage over cows' milk protein formulae and contain high concentrations of phytate, aluminum, and phytoestrogens (isoflavones), which might have untoward effects. There are no data to support the use of soy protein formulae in preterm infants. Indications for soy protein formulae include severe persistent lactose intolerance, galactosemia, and ethical considerations (e.g., vegan concepts). Soy protein formulae have no role in the prevention of allergic diseases and should not be used in infants with food allergy during the first 6 months of life. If soy protein formulae are considered for therapeutic use in food allergy after the age of 6 months because of their lower cost and better acceptance, tolerance to soy protein should first be established by clinical challenge. There is no evidence supporting the use of soy protein formulae for the prevention or management of infantile colic, regurgitation, or prolonged crying. ***JPGN 42:352–361, 2006.*** **Key Words:** soy—infant formula—follow-on formula—food allergy—phytoestrogens. © 2006 Lippincott Williams & Wilkins

INTRODUCTION

Soy formula was first introduced in the United States for feeding young infants in the early 1900s (1). In 1929, soy formula was proposed as a cows' milk substitute for babies with cows' milk intolerance (2). Soy protein formulae are given at some time during the first year of life to approximately 25% of infants in the United States, 13% in New Zealand, 7% in the United Kingdom, 5% in Italy, and 2% in France (3–6).

During the past few years, concerns have been raised over potential risks of soy protein formulae, in particular with regard to high phytoestrogen contents. Authorities or pediatric societies from Australia, Canada, France, Ireland, New Zealand, Switzerland, and the United Kingdom have recently advised health professionals and caregivers that because of concerns raised and limited availability of data, the use of soy protein formulae in infants should be restricted to specific cases (7–9).

The purpose of this comment by the Committee is to review available information on the composition and use of soy protein formulae as substitutes for breastfeeding and cows' milk protein formulae as well as on their suitability and safety for supporting adequate growth and

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development of infants. In preparing this comment, the Committee reviewed expert consensus documents on the use of soy protein formulae in dietetic products for infants (5,7–13). Products that do not meet the standards of infant and follow-on formulae or foods for medical purposes designed for infants, such as soy "milks" or juices and fermented soy products, that do not fulfill nutritional requirements of infants are beyond the scope of this review.

FROM SOYBEANS TO SOY PROTEIN ISOLATE FORMULAE

Soybeans comprise approximately 40% proteins, 35% carbohydrates, 20% fat, and 5% minerals (percent dry weight). Soybean products include oil and soy flour obtained from roasted soybeans ground into a fine powder. Soy protein isolates are derived from delipidated soy flour (90–95%) by elimination of soluble carbohydrates and mineral salts (5). Soy protein has a lower biologic value than cows' milk protein. The nitrogen conversion factor, which allows us to calculate the protein content from the total nitrogen content, is lower for soy protein isolate than for cows' milk protein. Soy and cows' milk proteins have a different amino acid pattern (i.e., soy protein contains lower amounts of methionine, branched chain amino acids lysine, and proline and higher quantities of aspartate, glycine, arginine, and cystine than cows' milk protein) (14). To ensure adequate growth, nitrogen balance, and plasma albumin concentrations, methionine supplements have been recommended (15,16). Because soy based products have a very low content of L-carnitine that may induce low plasma carnitine concentrations in infants (17), the addition of carnitine to soy formulae has also been recommended (7,18).

COMPOSITION OF SOY PROTEIN INFANT AND FOLLOW-ON FORMULAE

Recommendations and Regulations

The ESPGHAN Committee on Nutrition published recommendations on the composition of soy protein infant and follow-on formulae in 1990 (16). Soy protein infant and follow-on formulae marketed in the European Union must meet the compositional criteria defined by EU directives (19,20). For soy protein infant formulae, only protein isolates should be used, and the minimum protein content required by European legislation is higher than that of cows' milk protein infant formulae (2.25 g/100 kcal vs. 1.8 g/100 kcal) to account for potentially lower digestibility and therefore lower bioavailablility of soy protein compared with intact cows' milk protein. The main differences in compositional criteria between soy protein and cows' milk protein infant formulae, and between soy protein and cows' milk protein follow-on formulae, are listed in Table 1.

Nutritional Adequacy of Soy Protein Formulae

In the 1970s, Fomon et al. (21) studied infants fed, as desired, an infant formula based on methionine supplemented soy protein isolate with a protein content of 1.64 g/100 kcal and an energy content of 67 kcal/100 mL. Infants were fed the formula exclusively for 28 days and thereafter combined with complementary feeding until the age of 112 days. The infants had a similar growth pattern and similar normal markers of plasma protein metabolism as breast-fed infants. However, energy intakes were slightly higher than in infants fed a cows' milk formula with a protein content of 1.77 g/100 kcal. In a study designed to estimate the requirement of sulfur amino acids of infants up to the age of 112 days, a beneficial effect of L-methionine supplementation (7.5 mg/100 kcal) on nitrogen balance was only seen with a concomitant soy protein content of 1.8 g/100 kcal. A beneficial effect of methionine supplementation on weight gain or serum concentrations of urea nitrogen and albumin was only demonstrated at soy protein concentrations of 2.2 and 2.6 g/100 kcal, respectively (22).

Fomon et al. and other investigators demonstrated that infants exclusively fed methionine-supplemented soy protein formulae during the first 4 to 12 months of life showed weight gain and linear growth similar to that of infants fed conventional cows' milk protein formulae

TABLE 1. *Compositional criteria of soy protein isolate infant and follow-on formulae, alone or mixed with cows' milk protein, according to the Commission Directive 91/321/EEC of May 14, 1991 on infant formulae and follow-on formulae (19)*

	Soy protein infant formulae		Soy protein follow-on formulae	
	Minimum (/100 kcal)	Maximum (/100 kcal)	Minimum (/100kcal)	Maximum (/100 kcal)
Protein (g)*	2.25	3.0	2.25	4.5
Methionine (mg)	29	–	29	–
L-carnitine (μmoles)	7.5	–	–	–
Lactose (g)†	3.5	–	1.8	–
Iron (mg)	1	2	1	2
Zinc (mg)	0.75	2.4	0.75	–

*Soy protein isolate has to have a minimal chemical index of at least 80% in comparison with human milk protein for infant formulae and in comparison with human milk or casein for follow-on formulae.

†There is no minimal content for lactose when soy protein represents more than 50% of total protein.

(23,24). Studies were generally less than 1 year in duration, with exclusive soy protein formula feeding from birth to 4 months. Blood markers of protein metabolism in children fed soy protein formulae were not significantly different from those of infants fed cows' milk formulae. Healthy term infants fed a soy protein formula during their first year of life achieved a bone density similar to breast-fed or cows' milk formula fed infants (25,26). Outcome parameters included serum calcium, magnesium, phosphorus, alkaline phosphatase, parathyroid and 1,25-dihydroxyvitamin D concentrations, and bone mineral content measured with absorptiometry. These data indicate that soy protein formulae can be used for feeding term infants but have no nutritional advantage over cows' milk protein formulae.

In a randomized, controlled study performed in very low birthweight infants from 3 to 8 weeks of age, Hall et al. (27) compared a soy protein infant formula supplemented with calcium, phosphorus, and vitamin D (n = 17) with a whey-predominant premature infant formula (n = 15). Birth weight (1,206 ± 178 g) and gestational age (30 ± 1.9 weeks) of the soy formula-fed group were not significantly different from the whey formula-fed group (1,143 ± 158 g and 30 ± 1.8 weeks, respectively). The energy content of the whey formula was higher than that of the soy formula (81 kcal/100 mL vs. 67 kcal/100 mL), whereas the protein/energy ratio was identical in both formulae (3 g/100 kcal). The caloric (kcal/kg/day) and protein (g/kg/day) intake was not significantly different between each group because a greater volume of feed was consumed in the soy formula-fed infants. Those fed soy formula had lower weight gain (11.3 ± 2.3 g/kg/day) than infants fed whey-predominant formula (15.3 ± 2.5 g/kg/day) as well as lower protein and albumin blood concentrations. Bone mineralization pattern was the same in both groups. Although no more information is available in this population, the Committee concludes that soy protein formulae should not be used in preterm infants.

Phytate

Soy protein isolate contains some 1% to 2% phytate, which may impair the absorption of minerals and trace elements. In experimental animals and in human adults, phytate has a negative effect on intestinal zinc and iron absorption (28). A reduction in phytate contents of soy protein formulae can be achieved by precipitation methods or treatment with phytase. Reduction of the phytate content of soy formula increased the absorption and availability of zinc and copper in infant rhesus monkeys and rat pups and of iron in infants (29,30). Using stable isotope techniques in infants fed a soy protein isolate formula with low contents of phytate (<6 mg/kg liquid formula) or a conventional content (300 mg/kg liquid formula), Davidsson et al. (31) showed that zinc absorption was significantly greater with dephytinized formula (22.6% vs. 16.7%, $P = 0.03$), whereas no significant difference was observed for calcium, iron, copper, and manganese absorption.

Phytate may also interfere with iodine metabolism. Before the supplementation of soy formulae with iodine and the use of isolated soy protein instead of high-fiber soy flour in the mid-1960s, cases of goiter and hypothyroidism were described in infants fed soy formulae (32,33). The persistence of thyroid insufficiency despite the use of a high dose of levothyroxine has also been observed more recently in infants with congenital hypothyroidism fed soy protein formulae (34,35). A recent study showed that infants with congenital hypothyroidism fed soy protein formulae had a prolonged increase of thyroid stimulating hormone (TSH) when compared with infants fed nonsoy formulae. These infants need close monitoring of free thyroxine and TSH measurements and may need increased levothyroxine doses to achieve normal thyroid function (36). The mechanism of the prolonged increase in TSH blood concentrations is not clear. Malabsorption and increased fecal loss of the supplemented levothyroxine have been shown in animal studies performed before the use of isolated soy protein. Soy protein may also act as a goitrogen. A glycopeptide isolated from soy that blocks iodine uptake and decreases its organification has been described.

Information on the phytate contents of soy protein formulae used in Europe is not publically available. Such information should be disclosed by manufacturers. In view of the considerations discussed above, the Committee strongly recommends that phytate contents in soy protein infant formulae should be effectively reduced, for example, by precipitation methods or phytase treatment.

Nucleotides

The nucleotide content of soy protein formulae is much higher (approximately 310 mg/L) than that of human milk (68–72 mg/L) or cows' milk infant formulae (8–72 mg/L) (37). The Commission Directive 1991/321/EEC has approved the addition of nucleotides to infant and follow-on formulae with a total concentration of up to 5 mg/100 kcal, which is similar to reported data for free ribonucleotides in human milk (approximately 4–6 mg/100 kcal) (19). Because there is no adequate scientific basis at present to conclude that the addition of nucleotides in higher concentrations would provide additional benefits, the Committee discourages the further addition of nucleotides to formulae based on soy protein isolates given their high natural contents.

Aluminum

In 1996, the Committee on Nutrition of the American Academy of Pediatrics (AAP) highlighted the potential risk of aluminum toxicity in infants and children related to the use of soy protein formula contaminated with

aluminum (38). The source of the aluminum is thought to be the aluminum equipment used during the production of soy protein isolates and the nature of mineral salts used in formula production (3). Much higher concentrations of aluminum were found in soy protein formulae (500–2,400 μg/L) than in cow's milk protein formulae (15–400 μg/L) and breast milk (4–65 μg/L). However, daily aluminum intake remained less than 1 mg/kg, which the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives in 1989 considered as the tolerable intake of aluminum (39). Infants fed formulae with the highest contents of aluminum (2.35 mg/L) at the time of the publication would receive an aluminum dose less than 0.5 mg/kg per day at feed intakes up to 200 mL/kg per day. There is inadequate information on the aluminum content of soy protein formulae. Such information should be made available by manufacturers. Although long-term consequences of higher levels of aluminum observed in soy formulae are unknown, continued efforts should be made by manufacturers to reduce the aluminum content of soy protein formula.

Phytoestrogens

Phytoestrogens represent a broad group of plant-derived compounds of nonsteroidal structure that are ubiquitous within the plant kingdom and have weak estrogen activity (9,40). They are present in beans in general and soybeans in particular. Lignanes and isoflavones are the major classes of phytoestrogens of interest from a nutritional and health perspective. The main compounds contained in soy protein-based foods are the isoflavones genistein and daidzein (41). Isoflavones can bind to estrogen receptors, interact with enzyme systems influencing estrogenic activity, and exert weak estrogenic activity (42). It has been suggested that isoflavones may have anticancer properties in animals (43,44) and in human adults (45,46). Isoflavones may contribute to the prevention of cardiovascular disease, breast cancer, osteoporosis, and menopausal disorders (47), and they have been proposed to slow progression of renal disease in adults (48).

Infant formulae based on soy protein isolates contain relatively high concentrations of isoflavones (49). Isoflavone content found in soy formulae commercially available in the United States, United Kingdom, New Zealand, and France ranges from 17.5 to 47 μg/mL and from 123 to 281 μg/g of milk powder, with a higher proportion of genistein than of daidzein (8,50–53). Concentrations of isoflavones were much lower in cows' milk and breast milk samples, ranging from 0.1 to 5 μg/L in cows' milk (54) and from 1.6 to 13.6 μg/L (U.S.) and from 0 to 32 μg/kg (U.K.) in breast milk, respectively (8,41). Isoflavone content of breast milk varies with mother's diet. Setchell et al. (41) estimated that infants aged 1 to 4 months would receive 6 to 12 mg/kg bodyweight per day of total isoflavones, whereas an adult consuming 57 to 85 g of soy-based products may receive 50 to 100 mg of total isoflavones (i.e., 0.7 to 1.4 mg/kg/d).

Glycosidic conjugates of isoflavones present in soy protein formulae are hydrolyzed by intestinal glucosidases to their aglucon form, then are absorbed, metabolized in the liver to glucuronide and sulphate conjugates, and subsequently excreted in urine. Short-term studies have shown that no more than 30% of the ingested dose of isoflavones are recovered in urine and feces (41). Knowledge on the bioavailability of isoflavones is still incomplete in young infants (41,52). In 4-month-old infants exclusively fed soy protein isolate formula, Setchell et al. found plasma total isoflavone concentrations ranging from 552 to 1,775 μg/L, with a mean concentration of 980 μg/L. Mean (SD) plasma concentration was 684 (443) μg/L for genistein and 295 (60) μg/L for daidzein. These values were significantly higher ($P < 0.001$) than the mean values for plasma total isoflavone concentrations in infants fed either cows' milk formula (9.4 ± 1.2 μg/L) or breast milk (4.7 ± 1.3 μg/L) (41,50). On a molar basis, isoflavones demonstrated weak estrogenic activity relative to physiologic estrogens, possessing between 1×10^{-4} and 1×10^{-3} of the activity of 17 β-estradiol (55).

Phytoestrogens given at the high dosage contained in soy-based formulae adversely affected development and neuroendocrine function in different animal species (7,41,56). Isoflavones were found to cause infertility in sheep, known as "clover disease" (57). In utero exposure of rats to high doses of genistein impairs the pituitary secretion of luteinizing hormone (58).

It has been hypothesized that phytoestrogens have the potential to increase thyroid binding globulin (8). Any such increase could transiently increase the binding capacity for thyroxine, thus lowering free thyroxine concentrations. However, there are no data to suggest that phytoestrogens acting by this mechanism produce clinical effects. A retrospective telephone recall epidemiologic study found that children with autoimmune thyroid disease were significantly more likely to have been fed soy formula in infancy (31% vs. 13% in infants without autoimmune thyroid disease) (59). There was no group difference in the frequency and duration of breast feeding. The aglucons of genistein and daidzein were demonstrated to inhibit the activity of thyroid peroxidase purified from porcine thyroid glands when present at concentrations of 1 to 10 μM, resulting in iodinated isoflavone compounds. The presence of at least 150 μM of iodine per liter in the incubation mixture completely protected against the isoflavone-mediated thyroid peroxidase inactivation (60).

Few data are available on the potential consequences of exposure to high doses of phytoestrogens in human infants on the later sexual and reproductive development. A three-fold increase in the number of patients with premature thelarche seen between 1978 and 1981 in Puerto

Rico led to further investigation in a case-control study (61). Onset of thelarche before 2 years of age was significantly associated with consumption of soy protein isolate based infant formula and of various meats. However, less than 20% of cases were soy formula fed, which points to the importance of additional causative factors.

Strom et al. (62) conducted telephone interviews in 811 adults aged 20 to 34 years who had participated as infants during the years 1965 to 1978 in comparative but not randomized feeding trials with soy protein based infant formula (n = 248; 120 males) or cows' milk protein formula (n = 563; 295 males). Outcome measures were self-reported: pubertal maturation, menstrual and reproductive history, height, weight, and education levels. The study did not include any direct measurements of hormone levels. Females previously fed on soy formulae had a lower prevalence of sedentary activities (8.9 ± 3.4 hours/wk vs. 9.6 ± 3.5 hours/wk, $P = 0.05$), whereas there was no difference for males. No statistically significant differences were observed between groups in either men or women for adult height, weight, pubertal development, and incidence of thyroid disease. Women fed soy formula in infancy experienced a slightly but significantly longer duration of menstrual bleeding (by 0.37 days; 95% confidence interval [CI]: 0.06–0.68), with no difference in self-assessed intensity of menstrual flow. They also reported greater discomfort with menstruation (unadjusted relative risk for extreme discomfort vs no or mild pain, 1.77; 95% CI, 1.04–3.00). Pregnancies were reported by 42% of women fed soy-formulae and 48% of women fed cows' milk formulae (NS). Outcomes of pregnancies were not different, and neither were there differences between the groups in the prevalence of cancer, hormonal disorders, sexual orientation, or birth defects in the offspring. No conclusions can be drawn on possible effects on fertility in men previously exposed to soy-based formulae, considering their relatively young age at the time of the follow-up study. Although exposure to soy formulae in this study did not appear to be responsible for major health or reproductive problems, more information is needed on potential long-term effects of phytoestrogens.

Yellayi et al. (56) showed that subcutaneous genistein injections in ovariectomized adult mice produced dose responsive decreases in thymic weight of up to 80%. Genistein injection caused decreases in relative percentages of thymic CD4+CD8− and double positive CD4+CD8+ thymocytes, providing evidence that genistein may affect early thymocyte maturation and the maturation of CD4+CD8− helper T-cell lineage. Dietary genistein at concentrations that produced serum genistein levels substantially less than those found in soy protein formula-fed infants produced marked thymic atrophy.

In infants fed soy protein formula from birth to 4 months, Ostrom et al. and Cordle et al. (63,64) did not find differences compared with a control group that was breastfed for 2 months or more at 6 and 12 months of age for the level of immunoglobulins (Ig)G and A, the titre of antibodies against diphtheria, tetanus, poliovirus, and *Hemophilus influenzae* b, as well as the count of lymphocytes B, T, and NK. The only significant difference was the higher percentage of $CD57^+$ NK cells in the control group at 12 months.

Information on the phytoestrogen content of soy protein formulae should be made available by manufacturers. Although studies in humans are lacking, on the basis of available data in animal models, the Committee recommends that the content of phytoestrogens in soy protein formulae be reduced because of uncertainties regarding safety in infants and young children.

COMMENTS ON POSSIBLE INDICATIONS FOR SOY FORMULAE

Severe persistent lactose intolerance and galactosemia

Severe persistent lactose intolerance, including severe mucosal damage and the rare cases of hereditary lactase deficiency (McKusick 223000) and classic galactosemia (galactose-1-phosphate uridyltransferase deficiency) (McKusick 230400), are indications for the use of lactose free soy formulae (65). It should be noted that some soy protein formulae contain raffinose and stachyose that are cleaved in the digestive tract under the action of bacterial galactosidases, leading to the liberation of 1,4 galactose that may contribute to elevated galactose-1-P values in erythrocytes of galactosemic patients (66).

Acute gastroenteritis

A meta-analysis of clinical trials on the use of formulae in the management of acute gastroenteritis concluded that lactose-containing diets do not need to be withdrawn in the vast majority of cases, whereas lactose free diets were beneficial in a limited number of cases with severe dehydration (67). An ESPGHAN multicentric study has shown that the early use of lactose containing cows' milk formula after oral rehydration does not aggravate or prolong diarrhea in well-nourished infants presenting with acute gastroenteritis and mild to moderate dehydration and has the advantage of preventing malnutrition (68). Therefore, switching from lactose-containing formula to lactose free formula such as soy formulae is not routinely recommended in acute gastroenteritis (10). Moreover, there are theoretical concerns regarding the introduction of a new protein source in the presence of increased mucosal permeability, with a potential increased risk of allergic sensitization (69,70).

Cows' milk allergy

Before the availability of therapeutic formulae based on cows' milk protein hydrolysates, soy formula was the only dietetic product available for feeding infants with cows' milk protein allergy. However, soy protein is also a common allergen. The identification and characterization of soybean allergens have identified fractions containing conglycinin (molecular weight 180,000 d) and glycinin (molecular weight 320,000 d) as probably the major allergens and trypsin inhibitor as the minor allergen responsible for soy protein allergy (71). Patients with soy protein allergy present with either acute symptoms within a few hours after soy ingestion (i.e., urticaria, angioedema, vomiting, diarrhea, or anaphylactic shock) or with chronic symptoms (i.e. chronic diarrhea and failure to thrive, malabsorption, and villous atrophy) (72,73). Symptoms usually resolve after elimination of soy from the diet.

Among infants with cows' milk allergy fed soy protein based formulae, some 30% to 50% were reported to present with concomitant soy protein allergy, with a higher frequency reported in nonIgE-mediated enterocolitis-enteropathy syndrome (71,74–76). A review of 2,108 infants with cows' milk protein allergy followed at 33 Italian pediatric gastroenterology units reported that 50% of these infants had received soy protein-based formulae as the substitute for milk containing formulae. Soy protein formulae were discontinued in 47% of cases overall, ranging from 53% of infants younger than 3 months of age to 35% of children older than 1 year of age (4). The reasons for this discontinuation were not given in the publication.

In 1983, the AAP Committee on Nutrition discouraged the use of soy formulae in the dietary management of infants with documented allergy to cows' milk protein (77). The AAP Nutrition Committee concluded in 1998 that infants with documented cows' milk protein-induced enteropathy or enterocolitis are frequently sensitive to soy protein and should not be given soy protein formula routinely, whereas it emphasized that most infants with documented IgE-mediated cows' milk protein allergy will do well when fed soy formula (3). In 1990, the ESPGHAN Committee on Nutrition considered that available data did not support the view that soy formula should be the preferred choice in case of suspected or proven adverse effects to cows' milk protein (16). A joint statement of the ESPGHAN Committee on Nutrition and the European Society for Pediatric Allergology and Clinical Immunology stipulated that, in general, formulae based on intact soy protein isolates are not recommended for the initial treatment of food allergy in infants, although a proportion of infants with cows' milk protein allergy tolerate soy formula (11). The AAP Nutrition Committee stated in 2000 that infants with IgE-associated symptoms of allergy may benefit from a soy formula, either as the initial treatment or instituted after 6 months of age after use of a therapeutic hydrolysate formula (12).

The exclusion of soy protein from the diet of infants with IgE-mediated cows' milk protein allergy has been a controversial issue for a long time. In 93 children aged 3 to 41 months with IgE-mediated cows' milk protein allergy, Zeiger et al. (78) found a prevalence of concomitant soy allergy of only 14% (Table 2); 3% of the cohort were under 6 months of age at the time of evaluation and challenge. Diagnosis of soy protein allergy in this study was assessed by double-blind, placebo-controlled food challenge response to soy, open challenge response under the direction of a physician, or history of more than one immediate anaphylactic reaction to an isolated ingestion of soy. These investigators regard soy formula as a safe alternative to cows' milk formula for the vast majority of children with IgE-mediated cows' milk allergy, particularly those shown to have negative responses to soy challenge at the time of introduction of soy formula (78).

Klemola et al. (79) recently reported that the presence of concomitant soy allergy in infants with cows' milk allergy is less frequent than previously thought (Table 2). They conducted a prospective, randomized study to evaluate the cumulative incidence of allergy or other adverse reactions to soy formula compared with extensively hydrolyzed formula up to the age of 2 years in infants with

TABLE 2. *Studies on prevalence of soy allergy in immunoglobulin (Ig)E-associated cows' milk allergy (CMA) (78) and incidence of allergy to soy formula (SF) and extensively hydrolyzed formula (EHF) in cow's milk allergy (79)*

Reference	Study design	Allocation concealment	Blinding	Intention-to-treat analysis	Completeness to follow-up	Participants
Klemola et al., 2002 (79)	RCT	No	Single-blinded	Yes	Yes	n = 170 (with CMA confirmed by DBPCFC or history of an anaphylactic reaction)
Zeiger et al., 1999 (78)	Cohort study	NA	NA	NA	NA	n = 93, with IgE-mediated CMA

DBPCFC, double-blind, placebo-controlled food challenge; NA, not applicable RCT, randomized clinical trial; RR, relative risk; CI, confidence interval.

confirmed cows' milk allergy. The parents suspected adverse reactions significantly more often in infants randomly assigned to the soy formula than in infants randomly assigned to the extensively hydrolyzed formula (28%; 95% CI 18–39% vs. 11%; 95% CI 5–19%, respectively; relative risk [RR], 2.48; $P = 0.006$). Physicians diagnosed adverse reactions more often with soy than with the extensively hydrolyzed formula (10%; 95% CI 4.4%–18.8% vs. 2.2%; 95% CI 0.3%–7.8%, respectively; RR, 4.50; $P = 0.031$). Adverse reactions to soy were similar in IgE-associated and nonIgE-associated cow's milk allergy (11% and 9%, respectively). Adverse reactions were more common in younger (<6 months) than in older (6 to 12 months) infants (5 of 20 vs. 3 of 60, respectively, $P = 0.01$).

The use of soy formulae may play a role in the etiology of peanut allergy. Evaluating data from the Avon longitudinal study, a geographic-defined cohort study of 13,971 preschool children, Lack et al. (80) showed that peanut allergy was independently associated with intake of soy milk or soy infant formula during the first 2 years of life (odds ratio 2.6; 95% CI 1.4–5.0), suggesting the possibility of cross-sensitization through common epitopes. Soy protein fractions have been shown to be homologous to major peanut proteins (81). It is likely that children with allergy to cows' milk are at increased risk for food allergies, and soy consumption in infancy is increased in response to these atopic disorders. Indeed, a history of allergy to cows' milk (reported prospectively at 6 months) was significantly associated with peanut allergy ($P = 0.03$). In their study assessing the long-term effects of soy protein formulae, Strom et al. (62) showed that, as adults, females who had received soy formula in infancy more frequently used antiallergic and antiasthmatic drugs (18.8% vs. 10.1%, $P = 0.047$), whereas males showed a similar but nonsignificant trend (15.8% vs. 10.2%, $P = 0.08$).

The Committee concludes that for treatment of cows' milk protein allergy, the use of therapeutic formulae based on extensively hydrolyzed proteins (or amino acid preparations if hydrolysates are not tolerated) should be preferred to that of soy protein formulae. Given the limited number of infants studied (78,79) and the higher reported rate of adverse reactions to soy protein in infants under 6 months of age (79), the Committee recommends that soy protein formulae should not be used in infants with food allergy during the first 6 months of life. If soy protein formulae are used for therapeutic use after the age of 6 months because of their lower cost and better acceptance, tolerance to soy protein should first be established by clinical challenge.

Prevention of Atopic Disease

The role of soy protein formulae for the prevention of allergic disease in healthy and at-risk infants has been controversial (76,82) and is not supported by evidence from controlled trials (83–87). A recent meta-analysis of five randomized and quasi-randomized clinical trials with appropriate methodology concluded that soy formulae do not prevent food allergy in high-risk infants (13). The joint statement of the European Society for Paediatric Allergology and Clinical Immunology Committee on Hypoallergenic Formulas and the ESPGHAN Committee on Nutrition did not support the use of soy protein formulae for the prevention of allergy in at-risk infants (11).

Infantile Colic and Regurgitation

Soy protein formulae have been widely used in the industrialized countries for symptoms such as infantile colic, regurgitation, or prolonged crying without any convincing evidence for efficacy (23). Controversial data on the use of soy formulae have been obtained in infants with severe infantile colic attributed to cows' milk protein allergy (88,89). One randomized clinical trial showed a mean weekly duration of colic symptoms of 8.7 hours during treatment with soy formula, as compared with 18.8 hours during the control periods (mean difference = 10.1; 95% CI 3.8–16.5) (90). If persisting colic is defined as weeks in which there were 9 or more hours of colic symptoms, then colic persisted in only 31.6% of infants during the soy formula periods as opposed to 94.7% during the control periods (RR 0.33; 95% CI 0.017–0.65). The other randomized clinical trial of soy protein formulae did not allow firm conclusions to be drawn because of methodologic drawbacks (91). The meta-analysis of Lucassen et al. (92) collected 27

TABLE 2. *(continued).*

Age (mo)	Intervention group	Control group	Outcomes	Results	RR (95% CI)
2–11	SF (n = 80)	EHF (n = 90)	Parents suspected adverse reaction to the study formula	SF vs. EHF: 28% (95% CI 18–39) vs. 11% (95% CI 5–19)	2.5 (CI not given)
			DBPCFC confirmed adverse reaction to the study formula	SF vs. EHF: 10%; (95% CI 4.4–18.8) vs. 2.2%; (95% 0.3–7.8)	4.5 (1.1–18.4)
3–41	NA	NA	Soy allergy	14% (95% CI 7.7–22.7)	

controlled trials on the effectiveness of diets, drug treatment, and behavioral interventions on infantile colic. Soy protein formulae were not effective when only trials of good methodologic quality were considered.

Ethical and Religious Considerations

Some parents (e.g., vegans) seek to avoid cows' milk based formulae for their infants for religious, philosophical, or ethical reasons. Soy protein infant formulae is an acceptable alternative for these families.

CONCLUSIONS

1. Cows' milk-based formulae should be preferred as the first choice for feeding healthy infants that are not fully breast fed.
2. Soy protein based formulae should only be used in specified circumstances because they may have nutritional disadvantages and contain high concentrations of phytate, aluminum, and phytooestrogens, the long-term effects of which are unknown.
3. Indications for soy formulae include severe persistent lactose intolerance, galactosemia, religious, ethical, or other considerations that stipulate the avoidance of cows' milk based formulae and treatment of some cases of cows' milk protein allergy.
4. The Committee recommends that the use of therapeutic formulae based on extensively hydrolyzed proteins (or amino acid preparations if hydrolysates are not tolerated) should be preferred to that of soy protein formula in the treatment of cows' milk protein allergy. Soy protein formula should not be used in infants with food allergy during the first 6 months of life. If soy protein formulae are considered for therapeutic use after the age of 6 months because of their lower cost and better acceptance, tolerance to soy protein should first be established by clinical challenge.
5. Soy protein formulae have no role in the prevention of allergic diseases.
6. There is no evidence supporting the use of soy protein formulae for the prevention or management of infantile colic, regurgitation, or prolonged crying.
7. Manufacturers should aim to reduce the concentrations of trypsin inhibitors, lectins, goitrogenic substances, phytate, aluminum, and phytoestrogens in soy protein formulae.

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