Life Sciences Research Office Report

Executive Summary for the Report: Assessment of Nutrient Requirements for Infant Formulas

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FOREWORD

The Life Sciences Research Office (LSRO) of the American Society for Nutritional Sciences provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in relevant areas of biology and medicine.

This report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), in accordance with provisions of Task Order #10 of Contract No. 223-92-2185. The Health Protection Branch of Health Canada was a cosponsor of the report and is officially recognized as a liaison to the FDA for the purposes of this study. LSRO recognized the important roles of the American Academy of Pediatrics, Committee on Nutrition (AAP-CON) and Food and Nutrition Board (FNB) of the Institute of Medicine, National Academy of Science, in providing professional advice on issues related to the topics of this report. Therefore, efforts were made to keep these organizations fully informed on the progress of this study.

The final report was prepared and edited by Daniel J. Raiten, Ph.D., Senior Staff Scientist/Project Leader, John M. Talbot, M.D., Senior Medical Consultant, and Janet Waters, M.S., Staff Scientist, with input from Gregory J. Downing, D.O., Ph.D., Senior Staff Scientist. The report is based on discussions of, and materials evaluated by, an ad hoc Expert Panel convened by LSRO. The members of the Expert Panel were chosen for their qualifications, experience, and judgement with due considerations for balance and breadth in the appropriate professional disciplines. Members of the Expert Panel and others who assisted in the preparation of the report are identified in Chapter XII.

This study was initiated in September, 1995. In a notice in the Federal Register of March 5, 1996 the FDA announced that, as a component of Task Order #10, FASEB was inviting data, information, and views bearing on the topic under study (Food and Drug Administration, 1996a). Accordingly, LSRO provided an opportunity for public oral presentations in an Open Meeting held on May 31, 1996, and for written submissions. Fourteen (14) individuals made oral presentations at the Open Meeting. Two hundred sixty-three (263) individuals and organizations have provided written submissions for consideration by the Expert Panel (FDA Docket No. 96N-0005). These individuals and organizations are listed in Chapter XIII. The LSRO wishes to express its appreciation to all individuals and organizations who have contributed materials for this study.

individuals in Chapter XII does not imply that the individual Panel members specifically endorse all statements in the report.

The final report was reviewed and approved by an LSRO Advisory Committee. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Officer, American Society for Nutritional Sciences.

This LSRO report does not necessarily reflect the opinion of the individual members of the ad hoc Expert Panel or the members of the American Society for Nutritional Sciences, a constituent society of the Federation of American Societies for Experimental Biology.

Michael Falk, Ph.D., Director, Life Sciences Research Office, September 15, 1998.

EXECUTIVE SUMMARY

As mandated by the Infant Formula Act of 1980 (IFA), the Food and Drug Administration (FDA) has responsibility for ensuring the safety and nutritional quality of infant formulas. Regulations for infant formulas contained in the Code of Federal Regulations (CFR) include specifications for minimum levels of 29 nutrients and maximum levels of 9 nutrients (21 CFR 101.00). These nutrient specifications were last revised in 1985. In addition to the need to review the knowledge about those nutrients currently listed, new research has provided information on nutritional needs of infants not available in 1985. It was concluded as a result of this new information that there was a need:

- To evaluate evidence relative to the inclusion of several minerals not currently listed in CFR 107.100. In 1989, the Food and Nutrition Board (FNB) of the National Academy of Sciences (NAS) published the 10th edition of the Recommended Dietary Allowances (RDA) that included revision of some of the previous RDAs for infants, added an RDA for selenium and proposed for the first time estimated safe and adequate daily intakes (ESADDI) for fluoride, chromium, and molybdenum. None of these minerals is currently listed in 21 CFR 107.100.
- To consider the advisability of adding long-chain polyunsaturated fatty acids (LCPUFA), nucleotides, lactoferrin, and several other nitrogenous compounds to infant formulas.
- To reconsider the use of the Protein Efficiency Ratio (PER) as a measure of protein quality of infant formulas; this has been reinforced by recent advances in technology and the understanding of the nutritional needs of infants.

To revise the CFR and address these and other issues, the FDA recognized the need for a review of scientific information on the nutrient needs of healthy term infants; and, therefore, asked the Life Sciences Research Office (LSRO) to prepare a state-of-the-art analysis of the scientific literature, with an emphasis on, but not limited to, research published since 1985, and to do this in consultation with expert scientists and professional organizations involved in the field of infant nutrition. LSRO was also asked to consider the positions of other authoritative bodies, both domestic and international, in performing this review and to make recommendations.

To assist in accomplishing this task, the LSRO convened an Expert Panel consisting of scientists with particular expertise in the area of infant nutrition (see Chapter XII). This report contains a review of the literature and the specific recommendations of the Expert Panel.

PRINCIPLES AND CONCEPTS USED TO DEVELOP RECOMMENDATIONS

The overriding precept guiding the Expert Panel in its deliberations was to provide recommendations for nutrient content of infant formulas that could serve as the sole source

of nutrition for term infants throughout the first year of life. An additional concern was that these recommendations would not result in the imposition of unnecessarily restrictive conditions on the infant formula manufacturers. The Expert Panel used the following principles and concepts to reach its conclusions and recommendations.

- The Expert Panel strongly endorsed breast feeding as the preferred source of nutrients for infants and, for a number of nutrients, used the amounts found human milk as a guide in establishing minimum and maximum levels. Nevertheless, human milk was not considered *a priori* the reference for making recommendations for the nutrient content of infant formulas. For example, it was considered acceptable to include various carbohydrates not present in human milk in infant formulas, and to recommend maximum values for micronutrients that were well above the values commonly found in human milk.
- Although recognizing differences in nutritional needs of infants between birth and one year of age and realizing that formulas are not the sole source of nutrients for most infants in the second six months of life, the Expert Panel concluded that these differences could be accommodated by one set of recommendations.
- The Expert Panel recognized that there are a variety of factors that are involved in the determination of safe and adequate levels of nutrients for infant formula, including essentiality, stability, history of use, safety, and toxicity. Some or all of these factors are relevant for each nutrient required to be present in infant formula. Therefore, with few exceptions, the Expert Panel recommended both a minimum and maximum value for each nutrient.

- For a number of substances listed in the Scope of Work, the Expert Panel recommended neither minimum nor maximum values because insufficient evidence was found to justify the inclusion of the substance. These substances included g-linolenic acid, myristic acid, cholesterol, specific monosaccharides and disaccharides, oligosaccharides, glutathione, glycine, glutamine, urea, lactoferrin, medium-chain triglycerides, chromium, molybdenum, docosahexaenoic acid, and arachidonic acid.
- The specification of a maximum value for fluoride, taurine, and nucleotides, in conjunction with a minimum value of zero, did not constitute an endorsement for the inclusion of that substance; but rather, a recognition of apparent safety at levels defined by the maximum. Additional rationale for each nutrient is provided in the "Conclusions and Recommendations" sections.
- The Expert Panel recommended that in any case in which major changes in product composition are made (e.g., new protein or fat sources and nutrients previously not included), thorough clinical testing ought to be undertaken and the results reported.
- In setting minimum and maximum levels, the Expert Panel considered each nutrient individually and, in addition, considered the possibility of interactions that would affect the availability and utilization of other nutrients.
- All recommendations for nutrient specifications were made on the basis of energy content, i.e., per 100 kcal. Because of considerations of potential renal solute load (PRSL), a range of permissible energy densities (expressed as kcal/dl) was also specified.
- The recommendations concern formulas "as fed" (i.e., after the stipulated dilution of powdered or concentrated liquid products) and apply throughout the shelf-life of the product.

In selecting evidence for evaluation, the LSRO emphasized, but did not limit the evidence reviewed, to studies published since 1983, the date of the last revision of the AAP-CON review on recommended nutrient ranges in infant formula (AAP-CON, 1985b). This review provided the basis for the last revision of the IFA (Amendment to Infant Formula Act. Subtitle A of Title IV, Public Law 99-570; "Drug Enforcement, Education, and Control Act of 1986").

APPROACHES TO SETTING MINIMUM AND MAXIMUM NUTRIENT LEVELS FOR INFANT FORMULAS

Minimum. There are distinctions between the issues to be addressed in setting nutrient specifications to be used in the establishment of labeling requirements for infant formulas and the development of recommended dietary allowances (RDAs) as set by the Food and Nutrition Board (FNB) of the National Academy of Sciences (NAS) (NRC, 1989). Rather than defining a dietary allowance (with due consideration to bioavailability and metabolic variability within the population) intended to meet the physiological requirement for the nutrients in question, the LSRO asked the Expert Panel for recommendations about the nutrient content of formulas that will not only meet the requirements for growth and development, but will also be safe in terms of limiting exposure to potentially toxic substances.

In several cases the Expert Panel arrived at minimum levels that, upon conversion to units/d from units/100 kcal, were essentially equivalent to the 1989 RDA values. However, in noting that the nutrient specifications developed were intended to reflect the amount of those nutrients in the formulas **as consumed**, the Expert Panel recognized that

there were other issues that needed to be considered, e.g., nutrient stability, potential nutrient interactions, and environmental factors. In addition, upon review of the data, the Expert Panel in some cases reached conclusions that did not support the FNB recommendations. Consequently, there were some instances where recommended values differed from the RDA.

The Expert Panel utilized the following six types of evidence/support (Fomon, 1993a), in no hierarchical order, for the estimation of the minimum for each nutrient evaluated:

- direct experimental evidence;
- analogy to breast-fed infants;
- metabolic balance studies;
- clinical observations of deficiency;
- extrapolation from experimental evidence relating to human subjects of other age groups; and
- theoretically based calculations.

For each nutrient, the Expert Panel attempted to use as many of these approaches as possible, checking one against the other for consistency. For certain substances, the Expert Panel did not recommend minimum values; in such cases, the rationale for the decision is presented in the chapter section entitled "Conclusions and Recommendations." The Expert Panel used the following priority of approaches:

- When direct experimental evidence was available (e.g., for protein) or there were convincing clinical observations relating to the requirement (e.g., for vitamin B_6), this information was used.
- In the case of nutrients for which there were adequate data on the relative absorption of the nutrient from human milk and infant formulas, the minimum content of the nutrient in infant formulas was selected to permit absorption from infant formulas equal per unit of energy intake to that obtained by breast-fed infants. Thus, the mean minus one standard deviation value for the nutrient concentration in human milk (generally expressed as units per liter) was converted to units per 100 kcal.

  The Expert Panel selected the mean minus one standard deviation value for human milk because this represents history of use for a large population in which deficiency of nutrients has not been reported. This value was specified as the minimum content of infant formulas in cases in which absorption from human milk and infant formula was similar. If there was a known difference in absorption of the nutrient from human milk and infant formulas, the appropriate adjustment in the value was made. This approach was used for calcium, phosphorus, iron and zinc.
- For some nutrients, the Expert Panel utilized the 5-kg infant model consuming 100 kcal/kg/d to serve as a means to convert nutrient intake values (e.g., mg/d) to formula content values (mg/100 kcal). The 5-kg model, or reference infant, was used in cases where the Expert Panel referred to the RDA or ESADDI values to address the minimum content of specific nutrients that should be provided in infant formula. These reference values are provided in units of daily intake. The NRC (1989) cites an average intake of 750 ml/d by breast-fed infants during the first six months of life. It is generally accepted that human milk has an energy density of 67 kcal/100 ml, so the average daily caloric intake is 502 kcal/d. Thus, a 5-kg infant would consume 100 kcal/kg/d. This value was then used to convert the RDA or ESADDI value to formula contents. For a 5-kg infant consuming 100 kcal/kg/d, the average energy consumption per day would be 500 kcal. As an example, the RDA for vitamin K is 5 mg/d for the first six months of life, therefore, 5 mg/d divided by 500 kcal/d results in a minimum of 1 mg vitamin K/100 kcal.

Maximum. Because infant formulas may serve as the sole source of nutrition for young infants, it is equally important that nutrients be provided at levels that are safe both in terms of potential toxicity and possible adverse interactions among nutrients (e.g., an imbalance in the levels of two minerals which might result in amounts of one that are insufficient to meet physiological needs of the infant). The following are criteria established by the Expert Panel relative to the establishment of maxima for infant formulas:

- scientific evidence of toxicity, i.e., documented lowest observable adverse effect level (LOAEL) and/or no observable adverse effect level (NOAEL), or the potential for adverse nutrient interactions in animals and/or humans;
- knowledge of safety or history of use (absence of evidence of toxicity);

· in the absence of any toxicological evidence, the Expert Panel based its recommendations for maxima on current usage in marketed formulas. The FDA provided data on nutrient content of infant formulas (FDA, 1992) and the Expert Panel selected the 90th centile as the reference point reflecting high current intake patterns[1]. In view of no evidence of toxicity at the 90th centile, a lower centile would impose limitations that did not seem reasonable. With few exceptions, the Expert Panel considered that a lower centile would provide an unnecessarily limited range between minimum and maximum levels for some nutrients. The Expert Panel recognized that this approach if repeated in subsequent revisions of the CFR would lead to progressively lower maximum values, but for onetime use was considered satisfactory. It was noted that in many cases the FDA data are not normally distributed. Consequently, the Expert Panel recommended that, contingent on availability, consideration be given to substituting the geometric mean + 2 SD for the 90th centile value.

· In cases where there were no FDA data to address the nutrient levels of current usage (selenium, inositol, carnitine, choline, taurine, nucleotides and nucleotide precursors), the Expert Panel established the maximum levels as equivalent to the upper levels found in human milk.

For certain substances, the Expert Panel did not recommend maximum values; the rationale for these decisions is presented in each case in the relevant sections of the report.

SPECIFIC RECOMMENDATIONS

The following is a presentation of the specific recommendations of the Expert Panel with a brief explanation of the rationale used to justify each conclusion. The reader is referred to specific sections in the body of the report for more comprehensive review and background materials for each nutrient/substance covered in the Scope of Work (see Chapter I). **Table ES-1** lists the specific recommendations of the Expert Panel.

ENERGY AND MACRONUTRIENTS

Energy

The Expert Panel recommended that energy density of infant formulas fall in the range of 63 to 71 kcal/dl. When energy density of a formula is low, infants must consume a large volume to meet energy needs, and when energy density is high, fluid intake is decreased and the margin of safety with respect to water balance is decreased. History of use of formulas with lesser or greater energy density than 63 to 71 kcal/dl is limited.

FAT

Total Fat

Minimum: The Expert Panel recommended a minimum fat content of infant formulas of 4.4 g/100 kcal (40% of total energy). The current CFR minimum value of 3.3 g/100 kcal for fat (30% of energy intake from fat for a fully formula-fed infant) has not been tested in a comprehensive manner and is considered too low. Further, because beikost (solid foods) is introduced during the latter part of the first year of life, and these foods are often low in fat (e.g., single grain cereals and fruits), the total diet of an infant consuming a formula providing 3.3 g fat/100 kcal plus these types of foods could result in a total intake providing substantially less than 30% of energy from fat. The minimum of 4.4g/100 kcal for fat represents an intermediate value between the current CFR specification and the modal value for fat content (5.4 g fat/100 kcal) of currently marketed formulas.

Maximum: The Expert Panel recommended a maximum fat content of infant formulas of 6.4 g/100 kcal (57.2% of total energy). With the proposed minimum for protein of 1.7 g/100 kcal (6.8 kcal/100 kcal) and proposed minimum for carbohydrate of 9 g/100 kcal (36 kcal/100 kcal), a maximum

value for fat may not, therefore, exceed 57.2 kcal/100 kcal, equivalent to 6.4 g/100 kcal.

Linoleic Acid

Minimum: The Expert Panel recommended a minimum all-cis linoleic acid content of infant formulas of 8% of total fatty acids. With a minimum fat content of 4.4 g/100 kcal, the minimum linoleic acid content is therefore 350 mg/100 kcal. Concentrations of linoleic acid in human milk vary widely as a reflection of maternal dietary intake, but values less than 8% of fatty acids are rarely reported. Currently marketed infant formulas provide more than 8% of fatty acids as linoleic acid.

Maximum: The Expert Panel recommended a maximum linoleic acid content of infant formulas of 35% of total fatty acids. With a maximum fat content of 6.4 g/100 kcal, the maximum linoleic acid content is therefore 2240 mg/100 kcal. The polyunsaturated vegetable oils used in the manufacture of infant formulas, i.e., corn, safflower, and soybean oils, contain abundant amounts of LA (usually between 45 and 70% of total fatty acids). Historically, infant formulas particularly corn oil-based formulas have contained LA levels well in excess of 35% of fatty acids without reported adverse effects. Moreover, this value (35% of fatty acids) is within the limits that have been reported for individual human milk samples.

a-Linolenic Acid

The 1985 CFR does not provide for minimum or maximum levels of ALA in infant formula. The Expert Panel based its decision about the establishment of specifications for ALA on evidence supporting the essentiality for ALA to serve as a precursor for n-3 LCPUFAs, particularly DHA. The Expert Panel used the following lines of evidence as the basis for its recommendation to add ALA to infant formulas:

· Diets that provide adequate amounts of LA but less than 0.5 % of total energy as ALA are known to result in deficient n-3 LCPUFA levels, especially for DHA, while levels of 22:4n-6 and 22:5n-6 are increased in the structural lipid components of the brain as well as in other organs.

· Studies in term-gestation animals have shown that a diet containing more than 0.7% of total energy provided as ALA leads to accretion and deposition of DHA in the developing brain and other organs.

· The deleterious effect of ALA deficiency on visual function has been documented in various animal species. In rats, diets deficient in ALA have been associated with abnormal electroretinograms. Monkeys fed ALA-deficient diets were found to have increased ERG peak latencies in both retinal rods and cones, and increased time for retinal recovery of the dark-adapted response, with a-wave responses being mostly affected. Visual acuity, as assessed by the preferential looking acuity method, was also reduced.

· In animal studies, formulas with ratios of LA/ALA in excess of 16:1 have been found to result in lower DHA and higher 22:5n-6 in tissue lipids. In these studies, the brain total lipid DHA to 22:5n-6 ratio was significantly lower in animals fed formulas with a high LA/ALA ratio, in which the ALA level was < 0.7% energy.

· Studies in term infants have shown that feeding formulas with high (320:1) LA/ALA ratios resulted in significantly reduced RBC membrane DHA levels. It has been suggested that these effects are the consequence of LA inhibiting the conversion of ALA to DHA by D6-desaturase. However, the Expert Panel concluded that, based on the available studies, it is not possible to provide a clear picture of all the potential interactions of the n-3 and n-6 precursors and the end products of the metabolizing pathway or the effects of the amounts of ALA compared to the LA:ALA ratio.

Minimum: The Expert Panel recommended a minimum a-linolenic acid content in infant formulas of 1.75% of fatty acids with the further stipulation

that the ratio of LA:ALA not exceed 16 to 1. With the minimum total fat content of 4.4 g/100 kcal, the minimum content of ALA is 77 mg/100 kcal, approximately 0.7% of energy. This recommendation is based on the essentiality of ALA as a precursor of the n-3 series of LCPUFAs. Several studies suggest that formulas providing ALA at levels below this may be associated with delayed development of visual function and possibly lower levels of DHA in the brain. The recommended upper limit for the ratio of LA:ALA (16:1) was intended to prevent an inappropriate combination of high LA content with low ALA content, which might interfere with the formation of longer-chain fatty acids of the n-3 series.

Maximum: The Expert Panel recommended a maximum content of ALA in formulas of 4% total fatty acids with the additional stipulation that the ratio of LA:ALA not be less than 6 to 1. With a maximum fat content of 6.4 g/100 kcal, 4% of fatty acids from ALA amounts to 256 mg/100 kcal. The maximum was based on the long history of use of formulas containing soy oil (soybean oils typically contain 6-9% ALA) as the source of unsaturated fatty acids. The recommended minimum ratio of 6 to 1 was intended to ensure that the combination of the minimum LA content with the maximum level of ALA will not interfere with production of longer-chain fatty acids of the n-6 series.

g-Linolenic Acid

The Expert Panel concluded that there is no demonstrated benefit of the addition of GLA to infant formulas at this time. The Expert Panel found no convincing data to indicate that the conversion of LA to GLA is a limiting factor in the n-6 elongation pathway.

Polyunsaturated Fatty Acids with More than 20 Carbon Atoms

The Expert Panel did not recommend the addition of arachidonic acid (AA) or docosahexaenoic acid (DHA) to infant formulas at this time. The Expert Panel considered the evidence available to be insufficient to warrant a recommendation that these or other polyunsaturated fatty acids with 20 or more carbon atoms be added to infant formulas. Although blood levels of AA and DHA have been shown to be lower in infants fed formulas without these fatty acids compared to breast-fed infants, the relationship between dietary intake of fatty acids and blood concentrations is well known and is not necessarily evidence of benefit or dietary essentiality. Consequently, the Expert Panel placed particular emphasis on studies of growth and neurodevelopment in which LCPUFAs had been added to infant formula. The results of these studies have been inconsistent. Many of the studies have involved small numbers of infants, have not been adequately adjusted for potentially confounding variables, and have not been continued for sufficient length to determine long-term significance or to assess results with standardized measures of infant development of known predictive validity. Furthermore, the Expert Panel recognized that LCPUFAs are powerful mediators of metabolism, and concluded that the potential short- and long-term effects of these substances have not been sufficiently characterized in infants. Moreover, the Expert Panel was concerned that the metabolic and nutritional effects of the various available sources of LCPUFAs have not been adequately addressed at this time.

The Expert Panel was aware that clinical and basic science studies on the effects of supplementing infant formula with AA and DHA are being pursued and enthusiastically endorsed these efforts. Because of the limitations of the existing data, and the expectation that much additional data will become available in the near future, the Expert Panel recommended that the question of requiring the addition of specific LCPUFAs to infant formulas be reassessed within five years.

Myristic Acid

The Expert Panel did not recommend addition of myristic acid to formulas for term infants as no known role for myristic acid as a dietary nutrient currently exists.

Medium-Chain Triglycerides

With the exception of certain exempt formulas for infants with impaired fat digestion or absorption, the Expert Panel found no justification for the addition of medium-chain triglycerides to formulas for term infants.

Trans-Fatty Acids

The Expert Panel recommended that hydrogenated oils, representing the major source of *trans*-fatty acids, not be used in the manufacture of infant formulas. While the specific effects of dietary *trans*-fatty acids remain to be elucidated, in light of concerns about potential short- and long-term deleterious effects and the absence of any known nutritional benefit from these substances, the Expert Panel concluded that prudence dictates the limitation of these substances (to the extent possible) in infant formulas.

Vitamin E

The Expert Panel recommended that the vitamin E content of formulas be based on the polyunsaturated fatty acid content.

Cholesterol

The Expert Panel did not recommend the addition of cholesterol to infant formulas. There was no convincing evidence of a beneficial short-term or long-term effect of adding cholesterol to infant formulas, and no evidence that the added cholesterol would be equivalent to the cholesterol in the human milk fat globule.

PROTEIN, AMINO ACIDS AND OTHER NITROGENOUS COMPOUNDS

Total Protein

Minimum: The Expert Panel recommended a minimum protein content of infant formulas of 1.7 g/100 kcal, and specified that this value refer to true protein (i.e., a-amino nitrogen x 6.25). The Expert Panel specified the minimum protein content in terms of a-amino nitrogen (true protein) rather than total protein to indicate the importance of considering only those protein sources that are the main sources of nitrogen for tissue deposition and growth. The inclusion of nonprotein nitrogen sources (or total protein) in the specifications for the minimum protein content could result in an insufficient amount to support normal growth. Furthermore, although the Expert Panel considered the minimum intake of 1.7 g/100 kcal of true protein from milk-based formula to be adequate, it recommended that new formulations providing protein levels at or near the minimum level require clinical testing to demonstrate efficacy.

Maximum: The Expert Panel recommended a maximum total protein content of infant formulas of 3.4 g of crude protein (i.e., total nitrogen x 6.25) per 100 kcal. The Expert Panel concluded that the current CFR maximum protein level was too high because there was no physiological reason to provide protein at the level of 4.5 g/100 kcal; this level provides insufficient safety with regard to water balance, and adverse effects on zinc nutriture may occur. In addition to concerns about water balance and micronutrient nutriture (e.g., zinc, vitamin B_6), the Expert Panel was concerned specifically about the impact of all nitrogenous compounds, i.e., true protein and non-protein nitrogen, on the potential renal solute load (see Chapter VI). Consequently, the maximum protein content was specified in terms of crude (total) protein.

Finally, because of the considerable variability in the data utilized to establish protein requirements for infants, the Expert Panel strongly recommended that research efforts be encouraged to provide more standardized methodologies for accurately assessing intake and changes in functional outcomes consequent to changes in dietary protein intake. These issues will become particularly critical as new sources of protein are developed and become

available for use in infant formulas.

Protein Quality

The Expert Panel recommended that the assessment of protein quality be based on an amino acid score with human milk as the reference protein, thus eliminating the PER as an index of protein quality for infant formulas.

In reaching these conclusions, the Expert Panel observed the following tenets:

- the principal nutritional function of food protein is to supply (essential) indispensable amino acids (IAA) and "nonspecific" nitrogen to meet physiological needs;
- a primary factor is the physiologically available concentration of IAA;
- the balance or pattern of amino acids from a particular source is a major consideration when evaluating protein quality.

Among the more critical problems identified by the Expert Panel in its evaluation of the PER were:

- the high requirement of weanling rats for sulfur-containing amino acids that are not representative of human infant requirements;
- because rats cannot metabolize lactose, adjustments have to be made to both the formula being tested and the casein control to maintain analogous protein, fat, carbohydrate, and energy levels. Consequently, the formula as tested in the rat bioassay is not the same as that consumed by human infants; and
- the proteins from liquid formulas must undergo lyophilization before use in the rat bioassay, and, consequently, are not the same as the formulas consumed by human infants.

Additional concerns about the PER identified by the Expert Panel included poor reproducibility, high cost, and lack of precision.

The rationale for recommending the amino acid score with human milk as the standard was based on the following:

- adequate growth and development are known to occur in infants provided the standard, human milk;
- plasma amino acid profiles of infants have been shown to reflect the amino acid composition of human milk; and
- the composition of human milk has been characterized.

In light of these factors and the limitations of the PER, the Expert Panel concluded that the amino acid scoring pattern of human milk is an accurate and appropriate standard for assessing the protein quality of infant formulas. Furthermore, the Expert Panel found no convincing evidence to support the use of any other alternative to the PER. The application of the amino acid scoring system is described below.

The Expert Panel used the data from four independent sources on the amino acid composition of human milk (**Table 5-1**) to generate mean levels of IAA needed to accommodate the Panel's recommended minimum protein level for infant formulas, i.e., 1.7 g true protein/100 kcal. These calculated mean amino acid levels represent the Expert Panel's recommended minimum levels for infant formulas. The maximum for each IAA was calculated as twice the minimum level. The Expert Panel included the additional caveat that, when adding an individual amino acid, the total must not exceed 1.5 times the minimum recommended level. For the purposes of assessing protein quality, the Expert Panel recommended that cysteine and methionine values be combined (25 + 33 = 58) and that the values for phenylalanine and tyrosine be combined (63 + 70 = 133). Summary of human milk values for indispensable amino acids (**from Chapter V, Table 5-1**).

A summary of the IAA composition of currently marketed infant formulas is presented in **Table 5-2**. The Expert Panel's recommended minimum and

maximum values for amino acids in infant formulas are presented in **Table 5-3**.

The Expert Panel expressed the following additional caveats regarding protein quality assessment:

- Based on the similarity of apparent digestibility of currently marketed formulas, (cow milk-based or isolated soy protein-based), a correction for protein digestibility is not required.
- The evaluation of protein quality on the basis of amino acid score is merely a preliminary requirement (as was the PER) and does not obviate the need for clinical testing.
- The Expert Panel was aware that an amino acid score ignores the importance of several amino acids that may be conditionally essential, and does not take into account the possible adverse effects of antinutritional factors (e.g., trypsin inhibitors in soy; gossypol in cottonseed; lysinoalanine, which may be formed during heat processing of all protein sources). Therefore, clinical testing of infant formulas is essential for new sources of protein or for major alterations in methods of processing. In considering the quality of protein from novel sources, the Expert Panel concluded that the amino acid scoring pattern alone would be insufficient to determine protein quality.

Along with the indispensable amino acid composition, the Expert Panel concluded that additional measures were needed to reflect the digestibility and bioavailability of the amino acids from these sources. The assessment of protein quality of novel sources is an issue closely aligned with the general consideration of novel proteins in infant formula. The Expert Panel recommended that the issue of protein quality assessment for novel protein sources be reviewed in conjunction with the general consideration of the use of novel proteins in infant formulas within the next five years. For a further discussion of the issues related to the use of novel substances in infant formulas see Chapter X.

INDIVIDUAL PROTEINS, AMINO ACIDS, AND OTHER NITROGENOUS SUBSTANCES

Lactoferrin

The Expert Panel did not recommend the addition of lactoferrin to infant formulas at this time. Although it is technically feasible to add bovine lactoferrin or transgenic human transferrin to infant formulas, bovine lactoferrin does not bind consistently to human lactoferrin receptors and has not been shown to increase iron absorption. The efficacy and safety of adding human lactoferrin to infant formulas has not been adequately evaluated.

Given the emerging knowledge of the biological importance of human lactoferrin in infant nutrition, the Expert Panel regarded the notion of lactoferrin supplementation as worthy of consideration. However, clinical studies will be essential to demonstrate the efficacy and safety of such addition. Consistent with this position, the Expert Panel concluded that any consideration of the use of commercial formula manufactured using cow milk with transgenic sources of human lactoferrin be preceded by thorough evaluation of the scientific evidence of its nutrient value and safety. For a further discussion of issues and recommendations about the testing of new proteins for infant formulas see Chapter X.

Carnitine

Minimum: The Expert Panel recommended a minimum carnitine content of infant formulas of 1.2 mg/100 kcal, a level similar to that found in human milk. Although the evidence that dietary carnitine is essential for the term infant is not convincing, biochemical changes are noted when infants are fed a carnitine-free diet and there are several anecdotal reports of abnormal clinical manifestations associated with diets low in carnitine. Infants nourished with soy protein-based formula with low carnitine content had lower plasma and urine carnitine levels and evidence of altered lipid

metabolism, but no significant differences in rates of growth compared with supplemented infants. The functional significance of these metabolic differences in normal term infants is not known.

Maximum: The Expert Panel recommended a maximum carnitine content of infant formulas of 2.0 mg/100 kcal, a value similar to the upper limit reported for human milk. The Expert Panel was unaware of any studies in which a NOAEL or LOAEL had been identified for carnitine exposure in infants. Consequently, in the absence of data the Expert Panel concluded that the maximum should be set at a level comparable to the upper ranges of carnitine concentrations reported for human milk.

Taurine

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

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

Nucleotides

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

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

Glutathione

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

Urea

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

Glutamine

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

Glycine

The Expert Panel did not recommend the addition of glycine to infant formulas. There was no convincing evidence of a dietary requirement for glycine.

Choline

Minimum: The Expert Panel recommended a minimum content of choline in infant formulas of 7 mg/100 kcal based on limited available information on the lower end of the range for the choline content of human milk. A dietary requirement for choline has been demonstrated for several species of mammals, but not for humans. Because of their greater rate of growth, it seems likely that the need of the infant for dietary choline may be greater than at other stages of development. Further studies will be required to refine our understanding of the choline requirement in infants.

Maximum: The Expert Panel recommended a maximum content of choline in infant formulas of 30 mg/100 kcal. This recommendation was based on an extrapolation from adult data on the safe level of intake and allows for potential age-related differences in metabolism.

Inositol

Minimum: The Expert Panel recommended a minimum content of *myo*-inositol in infant formulas of 4 mg/100 kcal. Although the essentiality of dietary *myo*-inositol for infants remains an open question, it is an essential nutrient for at least two other species of mammals. Until more data on the requirement for *myo*-inositol are available, it seems prudent to reaffirm the CFR value of 4 mg/100 kcal.

Maximum: The Expert Panel recommended a maximum content of *myo*-inositol in infant formulas of 40 mg/100 kcal. Although current usage data are not available, this value is near the upper limit reported for human milk.

POTENTIAL RENAL SOLUTE LOAD (PRSL)

Renal solute load refers to all solutes (of endogenous and of dietary origin) that require excretion by the kidney. Under nearly all circumstances, most of the renal solute load is of dietary origin and is closely related to the nitrogen and electrolyte content of the diet. Potential renal solute load (PRSL) refers to solutes of dietary origin that would need to be excreted in the urine if none were diverted into synthesis of new tissue and none were lost through nonrenal routes.

For normal, healthy infants, renal solute load is not a problem even if whole cow milk is fed. However, during illness, water losses are often increased because of fever and, most importantly, because intake of milk or formula is usually decreased; renal solute load may then become an important consideration. As reviewed in the report), both epidemiologic evidence and theoretically based calculations suggest that PRSL of about 39 mosmol/100 kcal puts the infant at risk of hypertonic dehydration during illness. An upper limit for potential renal solute load of 33 mosmol/100 kcal has been recommended and it is evident that the lower the PRSL, the greater the margin of safety with respect to water balance. The Expert Panel concluded that a maximum value for PRSL of 33 mosmol/100 kcal was compatible with reasonable maximum values for the nutrients that enter into the calculation of PRSL. (Chapter VI)

Calculation of PRSL

An approximation of PRSL that is close enough for all clinical purposes is as follows:

$PRSL = N/28 + Na + Cl + K + P_a$

where N = nitrogen (milligram), Na = sodium, Cl = chloride, K = potassium, and P_a = available phosphorus, with the units in mmol (or mosmol) and the factor, N/28 represents excretion of nitrogenous substances (with the assumption that the modal number of nitrogen atoms per molecule is 2).

The Expert Panel recognized that approximately 50% of the phosphate in soy protein isolates and, consequently, 30% of the phosphorus in soy protein-based formulas, is complexed as phytate. Most of the phytate-associated phosphorus is not absorbed and therefore does not contribute to the PRSL. Therefore, for the purposes of this report, the Expert Panel considered available phosphorus or nonphytate phosphorus (P_a) the measured total phosphorus, less the measured phytate-associated phosphorus.

The recommended maximum levels of component nutrients preclude a value for PRSL greater than 33 mosmol/100 kcal.

PRSL Resulting from Proposed Maximum Levels of Component Nutrients

	CFR maximum values[1]	Proposed Maximum Values	
	(mg/100 kcal)	(mg/100 kcal)	(mosmol/100 kcal)
Nitrogen	720[2]	540	19.4
Na^+	60	50	2.2
Cl^-	150	160	4.6
K^+	200	160	4.1
P_a[-3]	-	70	2.3
			32.6

[1] Currently defined CFR maximum values.

[2] Refers to the crude protein/6.25.

P_a[-3] Refers to available phosphorus; assumed to be all of the nonphytate phosphorus of currently marketed cow-milk-based formulas and 70% of the

phosphorus in soy-based formulas.

CARBOHYDRATE

Total Carbohydrate

Minimum: The Expert Panel recommended a minimum total carbohydrate content of infant formulas of 9 g/100 kcal. This minimum was based on a theoretical calculation taking into account the amount of glucose needed for obligatory central nervous system oxidation while minimizing the contribution of gluconeogenesis. The calculation was based on the estimated needs for brain oxidation of 8 to 12 g/kg (at an estimated glucose utilization rate of 27 mmol glucose/100 g brain/min). Using this estimate, the 5-kg infant model consuming 500 kcal/d would require between 8 to 12 g glucose/100 kcal. The Expert Panel selected 9 g/100 kcal as a reasonable target within that range that would also accommodate the needs of infants up to one year of age (using the same utilization rate, the older infant would require 6.5 to 8.5 g/100 kcal).

Maximum: A maximum of 13 g/100 kcal was recommended for total carbohydrate in infant formulas. This value was arrived at by subtracting from 100% of the total energy (63 to 71 kcal/dl), the minimum energy provided by protein (1.7 g protein/100 kcal = 6.8 kcal) and the minimum energy from fat (4.4 g fat/100 kcal = 39.6 kcal) resulting in a maximum of 53.6 kcal from carbohydrate, equivalent to 13.4 g/100 kcal. This value has been rounded off to 13 g/100 kcal.

MONOSACCHARIDES

Glucose

The Expert Panel did not recommend the addition of glucose to infant formulas. The Expert Panel concluded that the inclusion of glucose to infant formula offered no biological advantage over other carbohydrate sources and would unnecessarily increase the osmolality of the formula and would increase the likelihood of the Maillard reaction occurring during processing.

OLIGOSACCHARIDES

Because the role of milk oligosaccharides in infant health is not well defined, the Expert Panel concluded that there were insufficient data available at the present time to establish a minimum or maximum level of these substances in infant formulas.

DISACCHARIDES

Lactose

Although the Expert Panel concluded that lactose is safe and appropriate for use in formulas by most healthy term infants, the Panel was not compelled by the evidence to recommend either a minimum or maximum level for lactose. Recognizing that lactose may be used as the sole carbohydrate source, it should not be used at a level greater than the recommended maximum value for total carbohydrate (i.e., 13 g/100 kcal). Furthermore, establishing minimum requirements for lactose in formula would not be appropriate because some term infants may require a low-lactose or lactose-free formula.

Sucrose

The Expert Panel did not recommend a minimum or maximum content of sucrose in infant formulas. The Expert Panel, recognizing that sucrose is safe for addition to infant formulas and may be useful for the palatability of some formulas (e.g., protein hydrolysate-based formulas), concluded that the use of sucrose is appropriate in some circumstances. The upper limit is defined by the upper limit for total carbohydrate.

POLYSACCHARIDES

Glucose Polymers

Although the Expert Panel concluded that glucose polymers are safe and appropriate for use in formulas by most healthy term infants, the Panel was not compelled by the evidence to recommend either a minimum or maximum level for such substances. The amount of carbohydrate provided from glucose polymers in a formula should be within the lower and upper limits for total carbohydrate, as designated by the Expert Panel.

Modified Food Starches

The Expert Panel concluded that the inclusion of modified food starches in infant formulas involves toxicologic rather than nutritional concerns. The Expert Panel did not have expertise in this area and therefore made no recommendations.

MINERALS

Calcium

Minimum: The Expert Panel recommended a minimum calcium content for formulas for term infants of 50 mg/100 kcal. The mean minus one standard deviation value for calcium content of human milk has been estimated by Fomon & Nelson (1993) to be approximately 220 mg/L (33 mg/100 kcal). The mean absorption of calcium by breast-fed infants is 58% of intake and by formula-fed infants is 38% of intake (Fomon & Nelson, 1993). Therefore, to achieve calcium absorption equivalent to that of most breast-fed infants, an intake of 50 mg/100 kcal[2] is required.

Maximum: The Expert Panel recommended a maximum calcium content of formulas for term infants of 140 mg/100 kcal. Although recognizing that this value is less than the 90th centile of calcium content calculated by the FDA in its evaluation of currently available infant formulas, the Expert Panel concluded that the calcium content of soy-based formulas is a consequence of the high phosphorus content of these formulas and the need to add more calcium to comply with the CFR requirement that the calcium:phosphorus ratio be less than 1.1 to 1. With its recommendation that the

phosphorus content of infant formulas be based on available phosphorus rather than total phosphorus, the Expert Panel concluded that a maximum calcium content of 140 mg/100 kcal would be more appropriate.

Phosphorus

Minimum: The Expert Panel recommended a minimum content of available< phosphorus of infant formulas of 20 mg/100 kcal. From the data summarized by Fomon & Nelson (1993), the mean minus one standard deviation value for phosphorus in human milk is approximately 115 mg/L (17 mg/100 kcal). The mean phosphorus absorption from human milk is 85% of intake and from milk-based formulas is 80% of intake. Therefore, to achieve phosphorus absorption equivalent to that of most breast-fed infants, an intake of 18 mg/100 kcal[3] is required; this value has been rounded off to 20 mg/100 kcal. Because this minimum is available phosphorus, it applies to all formulas, including those containing appreciable amounts of phytate.

Maximum: The Expert Panel recommended a maximum content of available phosphorus in infant formulas of 70 mg/100 kcal with the stipulation that the ratio of calcium to available phosphorus must not be less than 1.1 to 1 and not to exceed 2 to 1. According to the FDA analyses, the 90th centile value for the phosphorus content of infant formulas is 97 mg/100 kcal. On the assumption that phytate phosphorus accounts for 30% of the total phosphorus in isolated soy protein-based formulas, the 90th centile for available phosphorus is 70 mg/100[4] kcal.

The Expert Panel had concerns regarding the possibility of hypocalcemia in the immediate newborn period that may occur as a result of the consumption of a formula in which the minimum calcium content (50 mg/100 kcal) would be combined with a maximum phosphorus content, giving a calcium to phosphorus ratio of 0.71 to 1. The Expert Panel, therefore, added the recommendation that the calcium to phosphorus ratio not be less than 1.1 to 1.

Similarly, to avoid the possibility that the maximum content of calcium would be combined in a formula with the minimum content of available phosphorus, the Expert Panel recommended that the ratio of calcium to available phosphorus must not exceed 2 to 1.

Magnesium

Minimum: The Expert Panel recommended a minimum magnesium content of infant formulas of 4 mg/100 kcal. Because no evidence exists of a difference in availability of magnesium between human milk or infant formulas or deficiency in breast-fed infants from well-nourished healthy mothers, the Expert Panel concluded that the mean minus one standard deviation value (26 mg/L or 4 mg/100 kcal) for magnesium concentration in human milk was an appropriate minimum value for infant formulas.

Maximum: The Expert Panel recommended a maximum magnesium content of 17 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Iron

Minimum: The Expert Panel recommended a minimum iron content for infant formulas of 0.2mg/100kcal. This recommendation is made in an attempt to set the minimum so that the quantity of iron absorbed per 100 kcal from an infant formula will be similar to the quantity of iron absorbed from an equivalent intake of human milk by the breast-fed infant. Based on a number of reports (Fomon, 1993e), the Expert Panel concluded that after the first few months of life the mean iron content of human milk was no greater than 0.06 mg/100 kcal (0.4 mg/L). Because comparable data on absorption of iron from human milk and infant formulas were not available, the ratio of erythrocyte incorporation of an iron isotope was used as a surrogate for iron absorption. Based on the studies of Davidsson et al. (1994b) and Abrams et al. (1997), a value of 12.3% was accepted as the erythrocyte incorporation

of iron from human milk, and based on the studies of Rios et al. (1975) and Fomon et al. (1997), a value of 3.3% was accepted as the erythrocyte incorporation of iron from infant formula. Thus, the ratio was found to be 3.7:1. With a mean content of iron in human milk of 0.06 mg/100 kcal, the equivalent iron content of infant formulas must be 3.7 times greater or 0.22 mg/100 kcal, which was rounded off to 0.2 mg/100 kcal.

The majority of the Expert Panel concluded that formula-fed infants will be at risk of iron deficiency if they are fed for most of the first year of life with a content of 0.2 mg/100 kcal) and do not receive medicinal iron supplementation or foods, such as meats, that provide appreciable amounts of iron available for absorption. The Expert Panel recognized that it is difficult to assure adequate iron intake through these means and therefore endorsed the use of iron-fortified formulas for infants of all ages. However, the Expert Panel also acknowledged that some authorities in pediatric nutrition support the availability of infant formulas with low iron content, believing that these may be preferable at least during the first four months of life. Because the scientific evidence is not complete and the argument over the advisability of feeding iron-fortified formulas during the early months of life is not yet resolved, the Expert Panel concluded that the option of feeding low iron formulas should be maintained.

Concerns about these recommendations were expressed in the minority opinion (see Appendix C-2). The opposing arguments cited the following lines of evidence to support a larger minimum iron content in infant formula (4 mg/L; 0.6 mg/100 kcal):

- by 9 months of age the prevalence of iron deficiency in breast-fed infants ranges from 6% to 30%, thereby mitigating against the use of human milk as a standard,
- the incidence of iron deficiency anemia is lower among infants fed iron-fortified formula than among breast-fed infants or infants fed low-iron formulas, and
- the lack of scientific support for claims of gastrointestinal intolerance of iron-fortified formulas.

Maximum: The Expert Panel recommended a maximum iron content for infant formulas of 1.65 mg/100 kcal (11 mg/L). The current CFR maximum value for iron of 3.0 mg/100 kcal (20 mg/L) was considered unjustified in the light of available data. Based on studies identified in the report, the Expert Panel concluded that there is little or no advantage to iron fortification at a level greater than 1.2 mg/100 kcal (8 mg/L) and permitting an "overage" of 0.45 mg/100 kcal (more than 35% above a target level of 1.2 mg/100 kcal) would result in a maximum value of 1.65 mg/100 kcal.

This maximum iron content, if coupled with the minimum zinc content (0.4 mg/100 kcal) results in an iron-to-zinc ratio of 4.1 to 1 which was considered by the Expert Panel unlikely to interfere with zinc absorption. Further, when coupled with the minimum copper level (0.06 mg/100 kcal), the maximum iron content would provide an iron-to-copper ratio of 27.5 to 1, which was considered to impose no risk of inducing copper deficiency.

Zinc

Minimum: The Expert Panel recommended a minimum zinc content of infant formulas of 0.4 mg/100 kcal. In the absence of maternal deficiency, zinc deficiency in exclusively breast-fed infants is exceedingly rare. The mean minus one standard deviation value for the concentration of zinc in human milk at one month postpartum is approximately 2.4 mg/L (0.4 mg/100 kcal). Although zinc absorption from human milk and whey-predominant infant formulas is similar, absorption from milk-based formulas that are not fortified with whey proteins is less than from human milk, and from soy-based formulas may be less than half that of human milk. At an assumed value of 25% absorption from soy-based formulas, an intake of 0.4 mg/100 kcal would result in a daily intake of 0.5 mg for the reference 5-kg infant consuming 100 kcal/kg. This level of intake is equivalent to the calculated requirement to meet growth and excretion losses in infants in the second six months of life. Consequently, a minimum zinc content of 0.4 mg/100 kcal should assure adequate zinc absorption from all formulas.

Maximum: The Expert Panel recommended a maximum zinc content of infant formulas of 1 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Manganese

Minimum: The Expert Panel recommended a minimum manganese content of infant formulas of 1 mg/100 kcal. The mean minus one standard deviation value for the concentration of manganese in human milk is about 3 mg/L (0.4 mg/100 kcal). Because less is known about the requirement for manganese than for most other nutrients, the Expert Panel considered it prudent to set the minimum at a somewhat higher level.

Maximum: The Expert Panel recommended a maximum manganese content of 100 mg/100 kcal for infant formulas. There is considerable evidence that exposure to high intakes of manganese can be toxic, but the highest oral NOAEL has not been established for human subjects or for nonhuman primates. The proposed maximum of 100 mg/100 kcal is significantly below the estimated LOAEL in adults for manganese in water and is far beyond the range likely to be encountered in milk-based formulas. The Expert Panel concluded that this level is above the content likely to be associated with isolated soy protein formulas. The manganese content of isolated soy protein generally ranges from 10 to 20 mg/g of product and nearly the same per gram of protein. With a maximum protein content of 3.4 g/100 kcal, the manganese content of soy protein-based formulas will range from 34 to 68 mg/100 kcal.

The Expert Panel recommended the establishment of the NOAEL for chronic oral administration of manganese in young animals (nonhuman primates or some other appropriate species).

Copper

Minimum: The Expert Panel recommended a minimum copper content of infant formulas of 60 mg/100 kcal (0.4 mg/L). Based on published reports, the mean minus one standard deviation value for copper concentrations in human milk during the first three months postpartum can be estimated to be about 220 mg/L (33 mg/100 kcal). The mean daily copper intake in exclusively breast-fed infants has been estimated to be 0.23 mg (approximately 46 mg/100 kcal for the 5-kg infant consuming 100 kcal/kg) over the first four months of life. Copper deficiency in healthy term exclusively breast-fed infants is rare.

Data on the relative absorption of copper by breast-fed infants and formula-fed infants are not available. In studies of suckling rats copper absorption from human milk was comparable to that from milk-based formulas and less from a soy-based formula. However, percent absorption is negatively correlated with intake and the intake was four times greater from the soy formulas than from human milk. The Expert Panel concluded that the current minimum copper content of 60 mg/100 kcal includes a margin of safety that provides an adequate intake accounting for potential differences in bioavailability even for infants fed soy-based formulas.

Maximum: The Expert Panel recommended a maximum copper content in infant formulas of 160 mg/100 kcal, based on the 90th centile of the FDA analyses of infant formulas. The Expert Panel also indicated that this maximum level of copper would avoid adverse interactions with other minerals such as iron and zinc, and is significantly lower than copper intake levels that have been associated with copper-associated liver disease.

Iodine

Minimum: The Expert Panel recommended a minimum iodine content of infant formulas of 8 mg/100 kcal. Because the iodine content of human milk is so influenced by maternal iodine exposure, the mean minus one standard deviation value might not be the best reflection of dietary requirement. The Expert Panel concluded that at this time the RDA provided the best standard for iodine content of infant formulas. To achieve the RDA for iodine (40 mg during the first six months of life), a 5-kg formula-fed infant consuming 100 kcal/kg/d would need to obtain 8 mg/100 kcal. No studies were found in which the efficacy of the current CFR minimum of 5 mg/100 kcal was evaluated in healthy term infants.

Maximum: The Expert Panel recommended a maximum iodine content of infant formulas of 35 mg/100 kcal. The intake of iodine considered safe for adults is 1000 mg/d, equivalent to 14 mg/kg/d for a 70-kg adult. The current CFR maximum of 75 mg/100 kcal (75 mg/kg/d for an infant consuming 100 kcal/kg/d) is believed by the Expert Panel to be too high. The value of 35 mg/100 kcal recommended by the Expert Panel falls at about the 75th centile of the FDA analyses of currently marketed infant formulas and may be the lowest value feasible at this time.

The Expert Panel recommended that the FDA enter into a dialogue with the infant formula industry to seek means of decreasing the iodine content of milk. The Expert Panel also recommended that some priority be given to establishing the highest no observed adverse effect level of chronic oral administration of iodine in nonhuman primates or in some other appropriate species.

Sodium

Minimum: The Expert Panel recommended a minimum sodium content of infant formulas of 25 mg/100 kcal.

Because of the high coefficients of variation of reported values for sodium concentration of human milk, the Expert Panel was reluctant to follow the procedure that it applied to many other nutrients whereby the mean minus one standard deviation value for the nutrient in milk was accepted as the minimum value. The RDA of 120 mg/d for infants less than six months of age was considered a reasonable value. For a 5-kg infant consuming 100 kcal/kg, the RDA is equivalent to 24 mg/100 kcal; this value was rounded off to 25 mg/100 kcal.

Maximum: The Expert Panel recommended a maximum sodium content of infant formulas of 50 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Potassium

Minimum: The Expert Panel recommended a minimum potassium content of infant formulas of 60 mg/100 kcal. The Expert Panel based this recommendation on the mean minus one standard deviation value of the potassium content of mature human milk, which is approximately 60 mg/100 kcal (400 mg/L). No data were found to suggest a difference in potassium bioavailability between human milk and infant formula.

Maximum: The Expert Panel recommended a maximum value for potassium in infant formulas of 160 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Chloride

Minimum: The Expert Panel recommended a minimum chloride content of infant formulas of 50 mg/100 kcal. The mean minus one standard deviation value for the chloride concentration of human milk is approximately 320 mg/L (48 mg/100 kcal), this value has been rounded to 50 mg/100 kcal. There is no evidence that chloride is less well absorbed from infant formulas than from human milk.

Maximum: The Expert Panel recommended a maximum chloride content of infant formulas of 160 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Selenium

Minimum: The Expert Panel recommended a minimum selenium content of infant formulas of 1.5 mg/100 kcal (10 mg/L). This value approximates the estimated mean minus one standard deviation value for the selenium concentration of human milk in countries in which selenium deficiency has not

been recognized in breast-fed infants. This recommendation also provides sufficient amounts of selenium to account for potential differences in bioavailability of the various forms of infant formula. Based on a conservative estimate of 50% for the relative bioavailability of selenium from soy versus human milk, this recommended level would be sufficient to meet the estimated requirement for infants.

Maximum: The Expert Panel recommended a maximum selenium content of infant formulas of 5 mg/100 kcal. This is similar to the upper limits of the selenium content of human milk. Assuming an intake of 100 kcal/kg/d, an intake of 5 mg/100 kcal is far less per unit of body weight than the intake associated with development of selenosis in adults.

Fluoride

Minimum: The Expert Panel recommended a minimum level of zero for the fluoride content of infant formulas. Although it is recognized that fluoride promotes dental health, there is a question about whether the benefit of fluoride intake during early infancy warrants the risk of dental fluorosis.

Maximum: The Expert Panel recommended a maximum fluoride content of infant formulas of 60 mg/100 kcal. This value corresponds to current usage. Because most of the fluoride in the diet of a formula-fed infant comes from water used in diluting concentrated liquid or powdered formulas, little would be accomplished by decreasing the fluoride content of infant formulas to less than 60 mg/100 kcal.

Chromium

The Expert Panel did not recommend a minimum or maximum chromium content of infant formulas. Although the Expert Panel was convinced of the essentiality of dietary chromium, it recognized that chromium is ubiquitous in the environment and that it is unlikely that an infant formula can be produced with a chromium content lower than that of human milk. With the exception of individuals receiving total parenteral nutrition, chromium deficiency has not been reported in infants. Further, the Expert Panel acknowledged that dietary chromium is poorly absorbed, is of extremely low toxicity, and toxicity from dietary sources has not been reported in humans.

Molybdenum

The Expert Panel did not recommend a minimum or maximum molybdenum content of infant formulas. Although the Expert Panel was convinced of the essentiality of dietary molybdenum, it recognized that molybdenum is ubiquitous in the environment and that it is unlikely that an infant formula can be produced with a molybdenum content lower than that of human milk. With the exception of individuals receiving total parenteral nutrition, molybdenum deficiency has not been reported in infants. Further, the Expert Panel acknowledged that dietary molybdenum is poorly absorbed, is of extremely low toxicity, and toxicity from dietary sources has not been reported in humans.

VITAMINS

Vitamin A

Minimum: The Expert Panel recommended a minimum preformed vitamin A content of infant formulas of 200 IU/100 kcal (60 mg RE/100 kcal). A concentration of 100 IU/100 kcal in human milk is considered to be at or above the requirement for the healthy breast-fed infant. Because of the presence of bile-salt-stimulated lipase in human milk, the availability of vitamin A esters (the major form of vitamin A in human milk and infant

formulas) may be higher from human milk than from formulas. To account for this potential difference, the Expert Panel recommended a minimum level of 200 IU/100 kcal.

Maximum: The Expert Panel recommended a maximum vitamin A content of infant formulas of 500 IU/100 kcal (150 mg/100 kcal). Although the 90th centile of the FDA analyses of infant formulas is 570 IU/100 kcal, the upper centiles of the FDA values presumably reflect overages in vitamin A content above the current CFR minimum of 250 mg/100 kcal. The Expert Panel concluded that an upper limit of 500 mg/100 kcal is more appropriate in view of the recommended minimum of 200 mg/100 kcal.

Vitamin D

Minimum: The Expert Panel recommended a minimum vitamin D content of infant formulas of 40 IU/100 kcal. Although the Expert Panel believed this level to be generous, it has been in effect for many years in nearly all industrialized countries and has eliminated nutritional rickets in formula-fed infants. The Expert Panel found no scientific justification to recommend a change in the level.

Maximum: The Expert Panel recommended a maximum vitamin D content of infant formulas of 100 IU/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Vitamin E

Minimum: The Expert Panel recommended a minimum vitamin E content of infant formulas of 0.5 mg a-tocopherol equivalents (a-TE) per gram of polyunsaturated fatty acids (PUFA), but no less than 0.5 mg a-TE/100 kcal. The Expert Panel based its recommendations on the mean minus one standard deviation value for vitamin E in human milk, and the absence of data to justify a change in the current CFR.

Maximum: The Expert Panel recommended a maximum vitamin E content of infant formulas of 5 mg a-TE/g polyunsaturated fatty acids, based on the 90th centile of the FDA analyses of infant formulas. The Expert Panel concluded that this maximum content of vitamin E is below the intake levels that would result in toxicity as interpreted from the review of animal data, adult toxicology, and reports of adverse effects in preterm infants.

Vitamin K

Minimum: The Expert Panel recommended a minimum content of vitamin K_1 in infant formulas of 1 mg/100 kcal. The Expert Panel recognized that in certain circumstances, particularly the lower ranges of estimated intakes of breast-fed infants of unsupplemented mothers, the levels of vitamin K_1 in human milk may be insufficient to maintain normal blood clotting homeostasis Therefore, rather than rely on concentrations in human milk (i.e., mean minus one standard deviation), the Expert Panel recommended that the minimum content of vitamin K_1 in infant formulas be sufficient to provide the RDA of 1 mg/kg body weight. In light of recent evidence of functional changes at intakes of 0.15 m g/kg/day, the Expert Panel concluded that the RDA provides a significant margin of safety. To provide an intake of 1 m g/kg body weight for a 5-kg infant consuming 100 kcal/kg/d, the minimum formula content of vitamin K_1 should be 1 mg/100 kcal.

Maximum: The Expert Panel recommended a maximum content of vitamin K_1 in infant formulas of 25 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Thiamin

Minimum: The Expert Panel recommended a minimum thiamin content in infant formulas of 30 mg/100 kcal. The Expert Panel considered the adequacy of thiamin intakes by the exclusively breast-fed term infant as the primary evidence to support its recommendations. The Expert Panel based its recommendations on the knowledge that thiamin concentrations increase from the early stages of lactation to a mean of 200 m g/L (30 m g/100 kcal; range 23 to 35 m g/100 kcal) in mature milk, and on the absence of evidence to indicate that thiamin deficiency occurs in exclusively breast-fed infants from well nourished mothers. An intake of 0.75 L/d of human milk containing 200 m g/L of thiamin would provide a thiamin intake of about 150 m g/d, which is equivalent to 30 mg/100 kcal for the 5-kg infant consuming 500 kcal/day. The Expert Panel found no reason to suspect that the formula-fed infant has a greater thiamin requirement than the infant fed human milk.

Maximum: The Expert Panel recommended a maximum content of thiamin in infant formulas of 200 m g/100 kcal based on the 90th centile of FDA analysis of currently marketed infant formulas.

Riboflavin

Minimum: The Expert Panel recommended a minimum riboflavin content in infant formulas of 80 m g/100 kcal. In the absence of any new data on the riboflavin requirement of infants, this recommendation was based on the current RDA of 400 m g/d (80 m g/100 kcal for a 5-kg infant consuming 100 kcal/kg/day). Additional support for this recommendation comes from the study of Roughead & McCormick (1990a) in which the mean minus one standard deviation value, using state-of-the-art methologies for assessment of total riboflavin content of human milk, was 570 m g/L (85 m g/100kcal). In addition, it was concluded that the studies indicating potential biochemical deficiencies in infants nursed by unsupplemented mothers raised concerns about the use of lower values of breast milk reported in the literature.

Maximum: The Expert Panel recommended a maximum riboflavin content in infant formulas of 300 m g/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Niacin

Minimum: The Expert Panel recommended a minimum niacin content in infant formulas of 550 m g/100 kcal. Using the RDA for infants less than six months of age (5 mg NE/d) as the starting point, the Expert Panel factored the contribution of the tryptophan content of formulas in the following calculations:

A 5-kg infant consuming 100 kcal/kg/d requires 1000 m g NE/100 kcal to meet the RDA. Based on the Expert Panel's estimated minimum tryptophan content of 26 mg/100 kcal (see Chapter V, section on Protein Quality), the tryptophan contribution would be about 433 m g NE/100 kcal (60 mg tryptophan = 1 NE; 26 mg tryptophan = 26/60 or 433 m g NE). Consequently, the amount of preformed niacin to meet the RDA would be 1000 - 433 or about 567 m g preformed niacin/100 kcal. The Expert Panel rounded this value to 550 m g preformed niacin/100 kcal for ease of calculations.

Maximum: The Expert Panel recommended a maximum niacin content in infant formulas of 2000 m g/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Vitamin B_6

Minimum: The Expert Panel recommended a minimum content of vitamin B_6 in infant formulas of 30 m g/100 kcal. Although deficiency manifestations have not been reported in infants fed formulas providing 100 m g of vitamin B_6 per liter (15 m g/100 kcal for a 5-kg infant consuming 100 kcal/kg/d), the Expert Panel indicated that an intake of 130 m g/d (26 m g/100 kcal for a 5-kg infant consuming 100 kcal/kg/d), may be required to achieve

adequate vitamin B_6 nutritional status. The value of 26 m g/100 kcal was rounded to 30 m g/100 kcal. At the recommended maximum protein content of infants formulas (3.4 g/100 kcal), a vitamin B_6 content of 30 m g/100 kcal results in an intake level of 8.8 m g of vitamin B_6 per gram of protein, a ratio well above the 5.6 m g/g protein estimated as necessary to prevent the development of deficiency manifestations.

Maximum: The Expert Panel recommended a maximum content of vitamin B_6 in infant formulas of 130 m g/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Vitamin B_{12}

Minimum: The Expert Panel recommended a minimum vitamin B_{12} content in infant formulas of 0.08 m g/100 kcal (0.54 m g/L; 0.4 m g/d). This recommendation is based on the mid range of mean vitamin B_{12} levels in human milk and represents an intake above that reported to be associated with biochemical evidence of vitamin B_{12} deficiency (i.e., < 0.37m g/d). This vitamin B_{12} content will provide a sufficient intake to meet the RDA from birth to six months (0.3 m g/d; 0.06 m g/100 kcal for a 5-kg infant consuming 100 kcal/kg/d) and provides an additional margin of safety.

Maximum: The Expert Panel recommended a maximum content of vitamin B_{12} in infant formulas of 0.7 m g/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Folic Acid

Minimum: The Expert Panel recommended a minimum folic acid content in infant formulas of 11 mg/100 kcal. This recommendation is based on the most reliable and valid data on total folic acid levels in human milk for which the mean minus one standard deviation value is 71 m g/L or 10.6 mg/100 kcal.

Maximum: The Expert Panel recommended a maximum content of folic acid in infant formulas of 40 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Pantothenic Acid

Minimum: The Expert Panel recommended a minimum content of pantothenic acid in infant formulas of 300 mg/100 kcal. The Expert Panel based this recommendation on the mean minus one standard deviation value for pantothenic acid levels in human milk, (1.9 mg/L; 284 mg/100 kcal), which was rounded off to 300 mg/100 kcal. No new evidence could be found to justify a change in the current specifications.

Maximum>: The Expert Panel recommended a maximum content of pantothenic acid in infant formulas of 1200 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.<

Biotin

Minimum: The Expert Panel recommended a minimum content of biotin in infant formulas of 1 mg/100 kcal. The Expert Panel based this recommendation on the mean minus one standard deviation value of the biotin levels in human milk (5 mg/L; 0.75 mg/100 kcal) from the recent report by Mock et al. (1997) rounded up to 1 mg.

Maximum: The Expert Panel recommended a maximum content of biotin in infant formulas of 15 mg/100 kcal based on the 90th centile of the FDA analyses of infant formulas.

Vitamin C

Minimum: The Expert Panel recommended a minimum content of ascorbic acid in infant formulas of 6 mg/100 kcal. This value is consistent with the RDA for vitamin C for infants from birth to six months of age of 30 mg (roughly equivalent to a mean concentration in human milk of 40 mg/L assuming the model of 750 ml/d intake) and is equivalent to 6 mg/100 kcal for a 5-kg infant consuming 100 kcal/kg/d.

Maximum: The Expert Panel recommended a maximum content of ascorbic acid in infant formulas of 15 mg/100 kcal. This value, which results in an intake of 15 mg/kg/d for an infant consuming 100 kcal/kg/d, is equivalent per unit of body weight to an intake of approximately 1 g/kg/d by a 70-kg adult, an intake not associated with adverse effects. The Expert Panel recommended that research be undertaken to establish the maximum NOAEL for vitamin C in infant animals of an appropriate species.

[1] During the course of its deliberations, the LSRO Expert Panel received from FDA a detailed analysis of some samples of 300 infant formulas. The data consisted of distribution and frequency values for each nutrient in table format. Each nutrient is presented as a percentage of the AAP-CON minimum value. For example, if the minimum value for a nutrient is 1 mg/100 kcal, this is equivalent to 100%. If a sample was found to have 1.5 mg/100 kcal, this would be reported as 150% of the AAP-CON value. For each nutrient, the 90th centile was identified from the distribution data. Back to Text

[2] This recommendation is based on the following calculations:

220 mg/L (mean minus one standard deviation) x .58 (% absorption from human milk)» 128 mg/L (from breast milk)

128 mg/L ¸ .38 (% absorption from formula)» 336 mg/L (amount needed to be comparable to human milk)

336 mg/L @ .75 L/d (intake for the model 5 kg infant)= 252 mg/d

252 mg/d ¸ 5 (for 500 kcal/d for the 5 kg infant)» 50 mg/100 kcal

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[3]This recommendation is based on the following calculations:

115 mg/L (mean minus one standard deviation) x .85 (% absorption from human milk)» 98 mg/L (from breast milk)

98 mg/L¸ 80 (% absorption from formula)» 122 mg/L (amount needed to be comparable to human milk)

336 mg/L @ .75 L/d (intake for the model 5 kg infant)=92 mg/d

92 mg/d ¸ 5 (for 500 kcal/d for the 5 kg infant)» 18 mg/100 kcal

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[4] Although ~53% of the total phosphorus in soy protein sources is complexed as phytic acid, the contribution of additional phosphorus from other constituents of soy protein-based formulas reduces the proportion of unavailable phosphorus in soy protein-based formulas to ~30% of the total phosphorus content. Back to Text