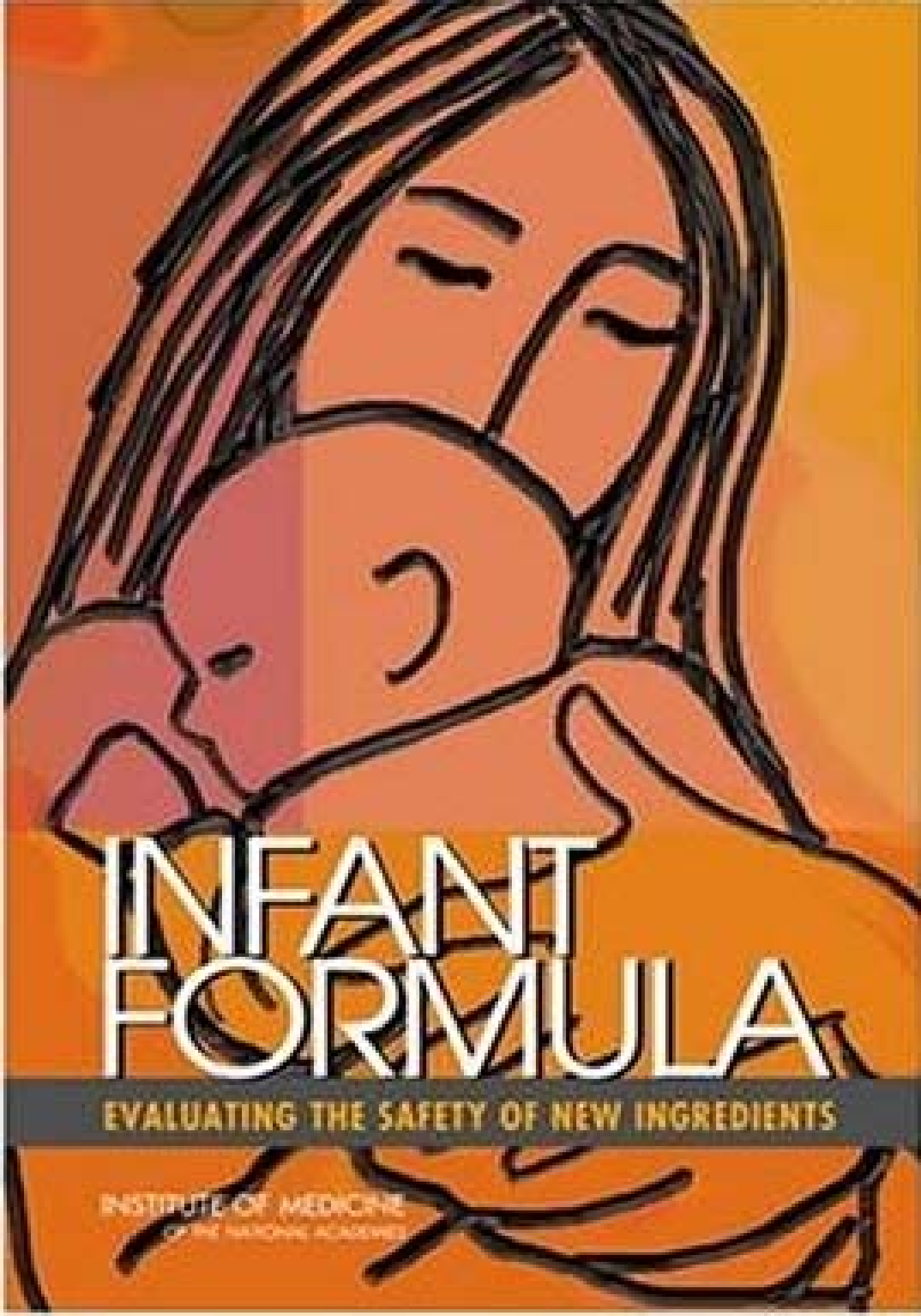

INFANT FORMULA

EVALUATING THE SAFETY OF NEW INGREDIENTS

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

INFANT FORMULA

EVALUATING THE SAFETY OF NEW INGREDIENTS

Committee on the Evaluation of the Addition of Ingredients
New to Infant Formula

Food and Nutrition Board

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.

THE NATIONAL ACADEMIES PRESS • 500 Fifth Street, N.W. • Washington, DC 20001

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This study was supported by Contract No. 223-01-2460, TO4, and Contract No. 4500033271 between the National Academy of Sciences and the Food and Drug Administration, U.S. Department of Health and Human Services, and Health Canada. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the organizations or agencies that provided support for this project.

Library of Congress Cataloging-in-Publication Data

Institute of Medicine (U.S.). Committee on the Evaluation of the Addition of Ingredients New to Infant Formula.

Infant formula : evaluating the safety of new ingredients / Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, Food and Nutrition Board.

p. ; cm.

Includes bibliographical references.

ISBN 0-309-09150-0 (pbk.)

1. Infant formulas—Standards—United States. 2. Infant formulas—Law and legislation—United States.

[DNLM: 1. Infant Formula—standards. 2. Evaluation Studies. 3. Food Analysis—methods. 4. Food Contamination—prevention & control. 5. Infant Formula—legislation & jurisprudence. 6. Infant Nutrition. WS 120 I59 2004] I. Title.

RJ216.I538 2004

613.2′083′2—dc22

2004008412

Additional copies of this report are available from the National Academies Press, 500 Fifth Street, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu.

For more information about the Institute of Medicine and the Food and Nutrition Board, visit the IOM and FNB home pages at **www.iom.edu** and **www.iom/fnb.edu.**

The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

Adviser to the Nation to Improve Health

COMMITTEE ON THE EVALUATION OF THE ADDITION OF INGREDIENTS NEW TO INFANT FORMULA

RICHARD J. DECKELBAUM *(Chair)*, Institute of Human Nutrition and Pediatric Gastroenterology and Nutrition, Columbia University, New York, New York
LINDA ADAIR, Department of Nutrition, Schools of Public Health and Medicine, University of North Carolina at Chapel Hill
MARK APPELBAUM, Department of Psychology, University of California, San Diego
GEORGE L. BAKER, Snowmass Village, Colorado
SUSAN S. BAKER, Department of Pediatrics, State University of New York at Buffalo
CHESTON M. BERLIN, Department of Pediatrics, Pennsylvania State University, Hershey
WILLIAM C. FRANKE, Center for Advanced Food Technology, Rutgers, State University of New Jersey, New Brunswick
MICHAEL K. GEORGIEFF, Department of Pediatrics, University of Minnesota, Minneapolis
JAMES M. NTAMBI, Department of Biochemistry, University of Wisconsin, Madison
THEODORE D. WACHS, Department of Psychological Science, Purdue University, West Lafayette, Indiana

Consultants
CARMI ZVI MARGOLIS, University Center for Health Sciences, Ben-Gurion University of the Negev, Israel
PEARAY OGRA, State University of New York at Buffalo
ODED SUSSKIND, Brookline, Massachusetts

Editor
SARAH CARROLL, Minnetonka, Minnesota

Staff
MARIA ORIA, Study Director (from June 2003)
PAULA TRUMBO, Study Director (September 2002–June 2003)
SANDRA SCHLICKER, Study Director (until September 2002)
LESLIE J. VOGELSANG, Research Assistant
SANDRA AMAMOO-KAKRA, Senior Project Assistant (from September 2002)
HARLEEN K. SETHI, Senior Project Assistant (until September 2002)

FOOD AND NUTRITION BOARD

CATHERINE E. WOTEKI *(Chair)*, College of Agriculture, Iowa State University, Ames
ROBERT M. RUSSELL *(Vice Chair)*, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts
LARRY R. BEUCHAT, Center for Food Safety, University of Georgia, Griffin
SUSAN A. FERENC, SAF*RISK, LC, Madison, Wisconsin
NANCY F. KREBS, Department of Pediatrics, University of Colorado Health Sciences Center, Denver
SHIRIKI KUMANYIKA, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia
REYNALDO MARTORELL, Department of International Health, Emory University, Atlanta, Georgia
LYNN PARKER, Child Nutrition Programs and Nutrition Policy, Food Research and Action Center, Washington, D.C.
BARBARA O. SCHNEEMAN, Department of Nutrition, University of California, Davis
NICHOLAS J. SCHORK, Polymorphism Research Laboratory, University of California, San Diego
JOHN W. SUTTIE, Department of Biochemistry, University of Wisconsin, Madison
STEVE L. TAYLOR, Department of Food Science and Technology and Food Processing Center, University of Nebraska, Lincoln
BARRY L. ZOUMAS, Department of Agricultural Economics and Rural Sociology, Pennsylvania State University, University Park

Staff
LINDA D. MEYERS, Director
GAIL E. SPEARS, Staff Editor
GERALDINE KENNEDO, Administrative Assistant
ELISABETH RIMAUD, Financial Associate

Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

M. Tom Clandinin, MTI MetaTech, Inc.; Susan Ferenc, SAF*RISK, LC; Kenneth Fisher, KD Consultants; Cutberto Garza, Cornell University; William C. Heird, Baylor College of Medicine; Charlie Homer, National Initiative for Children's Healthcare Quality; Nancy F. Krebs, University of Colorado Health Sciences Center; Mary F. Locniskar, Hill's Pet Nutrition, Inc.; Michael Posner, University of Oregon; Catharine Ross, Pennsylvania State University; Steve L. Taylor, University of Nebraska, Lincoln; John E. Vanderveen, San Antonio, Texas.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Dennis M. Bier, USDA/ARS Children's Nutrition Research Center at Baylor College of Medicine. Appointed by the Institute of Medicine, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committees and the institution.

Preface

Infant formulas are unique because they are the only source of nutrition for many infants during the first 4 to 6 months of life. They are critical to infant health because they must *safely* support growth and development during a period when the consequences of inadequate nutrition are most severe. The safety standard for an ingredient added to infant formula is defined as a "reasonable certainty of no harm." In recent years infant formula manufacturers have proposed ingredients (e.g., long-chain polyunsaturated fatty acids [LC-PUFAs], probiotics) that have created new scientific challenges that existing regulations may not address. Meanwhile the Food and Drug Administration (FDA) has been working to revise several aspects of its infant formula regulations, including requirements for quality factors and current good manufacturing practices.

In 2002 FDA and Health Canada asked the Institute of Medicine's Food and Nutrition Board (FNB) to formulate an expert committee to review and identify gaps in methods currently used to assess the safety of ingredients new to infant formulas. The committee was asked to identify tools to evaluate the safety of these ingredients under intended conditions of use in term infants. This charge included determining the data needed to demonstrate the safety of a component already present in human milk, that is, the effect of the matrix, and the utilization of preclinical and clinical studies and postmarket monitoring in the safety assessment process. FDA also asked the committee to apply its recommendations to the recent addition of LC-PUFAs as new ingredients in infant formulas, and others as appropriate.

A 10-member committee was appointed with expertise in the areas of pediatric nutrition, pediatric gastroenterology, epidemiology and public health, statistics, food technology, food regulatory processes, pediatric neurology, biochemistry, and infant formula manufacturing. Members brought a diversity of experience from research laboratories, industry, and hospital and clinic settings. Many of the committee members had never met, yet the group developed a cohesion that allowed them to work through and agree upon several difficult issues over the 18-month project.

The committee sought additional expertise and background from several consultants.

Two medical education consultants worked with the committee to develop several decision-making tools (algorithms) that emerged as important components of the committee's recommendations. An editorial consultant helped the committee bridge the gap from rough draft to final report to ensure uniformity of style and maximum readability for all possible users. Another consultant provided targeted guidance in the areas of immunology and endocrinology. In addition, the committee held two open sessions to learn more about updated systems and information that was relevant to its charge. In one open session, the committee reviewed differences that might apply to safety of foods as compared with drugs. In the other open session, one specialist discussed the use of growth charts and another from FDA's Center for Food Safety and Applied Nutrition discussed the Adverse Event Reporting System.

The committee held six meetings and utilized frequent conference calls to develop this report. The committee decided at an early stage that as a general approach, it would first review current (and past) approaches to establishing the safety of ingredients new to infant formulas. Next, gaps or limits of the current systems were to be identified, and then recommendations were to be proposed to improve current approaches. Realizing that detailed recommendations for every possible new ingredient would be impossible to achieve within the framework of the committee's work, generic templates were designed to be utilized (and modified) to fit a large range of potentially new ingredients with varying characteristics and different levels of safety concern.

The committee proposes the use of a hierarchical approach to assess the safety of ingredients new to infant formulas. These hierarchies, which often utilize algorithms, assist decision-making through a step-by-step process that places priority on commonly used assessments (e.g., screening) and progresses to more specific assessments (e.g., neuroimaging) when early indicators suggest safety concerns. The primary benefit of using algorithms is that they provide an appropriate balance of information and flexibility to navigate through a decision-making process without being prescriptive. This is especially useful given the wide diversity in possible new ingredients.

On behalf of the committee, I am grateful to FNB's staff for their support and contributions. This report was guided by three study directors: Sandra Schlicker laid the initial foundation for the committee's work, Paula Trumbo provided input through the middle stages of deliberation, and Maria Oria assisted the committee in bringing the report to its conclusion. The committee is grateful to Leslie Vogelsang, research assistant, and Sandra Amamoo-Kakra, senior project assistant, for their support and dedication. The committee would also like to thank Linda Meyers, director of FNB, for her objective insights and invaluable expertise that informed committee deliberations and conclusions and Allison Yates for her leadership during the early stages of the project. This report would not have been possible without the leadership and dedication of the FNB staff.

Finally, I wish to thank each member of the committee for his or her unique contributions and unfailing dedication to a report that has the potential to improve the safety of infant formulas for a new generation.

Richard J. Deckelbaum, *Chair*
Committee on the Evaluation of the Addition of
Ingredients New to Infant Formula

Contents

APPENDIXES

Executive Summary

OVERVIEW

Existing guidelines and regulations for evaluating the safety of conventional food ingredients (e.g., vitamins and minerals) added to infant formulas have worked well in the past; however they are not sufficient to address the diversity of potential new ingredients proposed by manufacturers to develop formulas that mimic the perceived and potential benefits of human milk. Proper nutrition, while important throughout life, is particularly critical during infancy when growth and development are most rapid and when the consequences of inadequate nutrition are most severe. Not all organ systems are fully mature at birth, and many are highly susceptible to environmental inputs as they undergo further development. Thus optimization of nutrition and minimization of exposure to potentially harmful substances in the food supply is of heightened importance during infancy.

Multiple health organizations endorse breastfeeding as the optimal form of nutrition for human infants because of its potential advantages to the infant, including prevention of infectious diseases and its role in neurodevelopment. Despite these recommendations, the vast majority of infants worldwide are fed infant formulas (e.g., liquid or reconstituted powders) at some point in their first year of life, whether as their sole source of nutrition or in combination with human milk, supplemental foods, or both. Infant formulas have been modified over the years to improve flavor, increase shelf life and, recently, to mirror the composition of human milk and the performance of breastfeeding.

Existing guidelines and regulations to assess the safety of ingredients new to infant formulas do not provide a clear and complete set of tools to address the new scientific challenges created by the addition of new ingredients (e.g., probiotics and other complex ingredients). For example, in the United States the Generally Recognized as Safe (GRAS) Notification is the most common approach that manufacturers use when they seek to add a new ingredient to infant formula. This process, while scientifically rigorous and transparent, was developed to regulate food ingredients for the general population, not for infants who are a more vulnerable population.

This report, prepared at the request of the Food and Drug Administration (FDA) and Health Canada (with potential international utility), addresses the regulatory and research issues that are critical in assessing the safety of the addition of new ingredients to infant formulas.

COMMITTEE CHARGE AND APPROACH

The Committee on the Evaluation of the Addition of Ingredients New to Infant Formula, convened by the Institute of Medicine, was asked to:

- review methods currently used to assess the safety of ingredients new to infant formulas,
- identify gaps in current safety assessment guidelines, and
- identify tools to evaluate the safety of ingredients new to infant formulas under intended conditions of use in term infants.

The committee was asked to focus on ingredients that are regulated under the *food* provisions of the law and to consider the health and well-being of *term* infants from birth to 12 months of age. This charge included determining which new ingredients or classes of ingredients are of lesser or greater concern, which additional data would be needed to demonstrate the safety of a component already present in human milk when it is added to the matrix of infant formula, the usefulness of certain safety tools and approaches, and the utilization of preclinical and clinical studies and in-market monitoring. Finally, the committee was asked to apply its recommendations to long-chain polyunsaturated fatty acids (LC-PUFAs), recently determined GRAS, as a new ingredient in infant formulas and to other ingredients as appropriate.

The committee reviewed U.S., Canadian, and European laws and regulations to examine current processes for manufacturers who wish to add new ingredients to infant formulas: a GRAS Notification and a Food Additive Petition. The committee drew on this review, especially the GRAS Notification process, as it developed its recommendations. The committee also reviewed the special needs of infants and their implications for evaluating the safety of infant formulas.

The committee developed and used algorithms throughout the report to graphically depict the overall process and recommends the use of stepwise decision-tree approaches for the process and for preclinical studies, clinical studies, and in-market surveillance. Each algorithm is a step-by-step decision tree that depicts the logic of a process but does not denote a particular chronology. Algorithms provide a useful tool and a visual way to explain the process of planning the type and depth of safety assessments; to improve data collection, problem solving, and decision making; to incorporate multiple levels of information into a single document; and to utilize a linear approach to identify critical information needed at major decision points. The committee also applied its recommended approaches to LC-PUFAs and to probiotics, recently determined GRAS, to examine the utility of the approaches.

The committee recognizes that some of its recommendations may require statutory changes. Even with this limitation, the committee encourages dialogue among members of government agencies, the public, industry, and academia to act on the recommendations set forth by this report in the best interest of our most vulnerable members of society—our infants.

FINDINGS AND RECOMMENDATIONS

Approach for Evaluating Safety

Safety evaluation of food ingredients in the United States is based on "reasonable certainty of no harm." Sections 201(s), 201(z), 409, and 412 of the Federal Food, Drug and Cosmetic (FD&C) Act, associated regulations, and FDA's *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, also known as the Redbook,[1] provide guidelines that are used to assess the safety of food ingredients and infant formulas. The Canadian Food and Drugs Regulations, administered by Health Canada, include specific requirements for infant food, novel food, and food ingredients.

In Canada and the United States, the food additive petition processes are similar and require premarket review and approval. In the United States, under the proposed GRAS Notification rule, a manufacturer can declare that a substance is GRAS if there is scientific consensus among qualified experts about its safety under the conditions of intended use. The manufacturer then notifies FDA, and if the agency has no questions, a letter of no objection is issued.

A primary difference between the two routes is that the Food Additive Petition places the responsibility of declaring that a substance is safe and approved under the conditions of use with the regulatory agency, whereas the GRAS Notification process places the responsibility of demonstrating that a substance is GRAS, and therefore safe under the conditions of use, with the manufacturer. The GRAS Notification and Food Additive Petition procedures are intended to ensure the safety ("a reasonable certainty of no harm"; FD&C Act Section 409), not the efficacy, of the proposed ingredient. The vast majority of new ingredients will likely follow the GRAS process to establish safety.

In addition to the regulations, FDA provides preclinical guidelines in its Redbook. This document was prepared to assist in the design of protocols for animal studies conducted to test the safety of food ingredients and includes detailed guidelines for testing the effects of food ingredients on mothers and their developing fetuses. However the special conditions surrounding infancy require distinct procedures to ensure the safety of infant formulas.

The GRAS process is rigorous, flexible, credible, and transparent. However its application does not clearly address possible concerns for the multitude of potential new ingredients in infant formulas. New ingredients may possess a variety of chemical characteristics, nutritional contributions, and pharmacological and physiological activities. Ingredients may be derived from novel sources or processes (e.g., products of fermentation or biotechnology). Such diversity requires safety guidelines that are clear but not overly prescriptive because of the disparity in the issues that each class of ingredient to be added to infant formulas may present. Because of its wide use, the committee used the GRAS Notification process as a starting model in developing its proposed approach for assessing the safety of new ingredients added to infant formulas, without being prescriptive. Some elements of the system proposed by the committee are currently in place.

Infant formula is the only source of nutrition for many infants and, therefore, additional safeguards have been established for infant formulas to which new ingredients are added that are not required for other foods. Regulations under Sections 201(s), 201 (z), and 412 of the FD&C Act have been implemented to ensure the safety of an infant formula to be marketed with a new ingredient. Under these sections of the Act, a proposed rule would

[1]This FDA document should not be confused with the American Academy of Pediatrics' Red Book of childhood infectious diseases.

mandate that manufacturers must demonstrate that the formula containing the new ingredient is capable of sustaining physical growth and development over 120 days when formula is likely to be the sole source of infant nutrition. The committee believes that data from growth and development studies should be submitted as part of the material demonstrating safety.

Important Safety Considerations When Regulating Infant Formulas

Most of the principles that govern the safety of ingredients new to infant formulas derive from the same principles that govern food safety for older children and adults. **The committee concluded that the six issues listed below must be considered as important safety issues when regulating infant formulas:**

- Infant formulas are the sole or predominant source of nutrition for many infants.
- Formulas are fed during a sensitive period of development and may therefore have short- and long-term consequences for infant health.
- Animals may not be the most appropriate model on which to base decisions of safety.
- "One size fits all" food safety models may not work for all new additions to formulas.
- Infant formulas could be considered as more than just food (i.e., as a delivery system for non-nutritional agents).
- Potential benefits, along with safety, should be considered when adding a new ingredient to formulas.

Use of a Hierarchical Approach

Since infants have distinct needs and vulnerabilities, a set of guidelines should be developed to provide a hierarchy of decision-making steps for manufacturers seeking to add new ingredients to infant formulas. Because specific safety assessments will need to be targeted according to the nature of the ingredient, the set of guidelines should allow for flexibility in the approach, while being rigorous and scientifically based.

A hierarchical approach assists in determining the appropriate level of assessment by considering: (1) the harm (e.g., toxicity), and (2) the potential adverse effects of a new ingredient. This hierarchical approach will guide the level of assessments to be applied to the new ingredient by considering the following factors:

- the reversibility of potential harmful effects,
- the severity and consequences of adverse effects,
- the time of onset of manifestations of the adverse effects,
- the likelihood that a new ingredient could adversely affect a specific system, and
- whether the effect would be common or rare.

This approach to evaluating the safety of new ingredients to be added to infant formulas was based on the uniqueness and vulnerability of the infant population. Therefore each step in the process requires empirical evidence from many disciplines and the application of the highest standards, whether using methods of bioassay, nutritional analysis, or basic chemistry. This approach is valuable in determining the relative importance of potential adverse effects for each specific new ingredient by providing generic templates for different steps in the safety assessment process rather than specific recommendations for each compound. It is neither realistic nor desirable to design individual templates for each new ingredient; rather,

expert panels can refine the generic templates as needed. This approach is designed for a broad spectrum of ingredients and could be applied to new ingredients to be added to infant formulas regardless of the regulatory process used.

The hierarchical approach is graphically presented by algorithms throughout the report and is applied in Appendix D to LC-PUFAs and probiotics. Each algorithm (see Figures ES-1 through ES-7) is a step-by-step decision tree that depicts the logic of the process but does not denote a particular chronology. For example, a manufacturer may initiate several different studies and procedures at the onset of the process, the results of which could be assessed at different steps in the algorithm. Any new ingredient considered for use in infant formulas must be considered in the context of its form, the matrix, and other ingredients with which it may interact.

Expert Panels

The committee recommends that manufacturers establish balanced, qualified expert panels in consultation with the regulatory agency. The existing GRAS process requires consensus by qualified experts to evaluate the safety of the ingredient under consideration; this consensus is often reached through a panel of scientific experts. The current system does not specify the composition of the panel, and manufacturers may be uncertain about the selection of appropriate panel members.

The panel should have experts that will ask the right questions and form an opinion that is robust and of the highest scientific integrity. The committee strongly recommends that the expert panel include a physician experienced in clinical study assessments, preferably a pediatrician. Guidelines for selecting a panel early in the process could improve the efficiency and objectivity of the process. Each expert panel should be responsible for determining the requisite levels of preclinical and clinical studies and in-market surveillance needed to ensure the safety of new ingredients by utilizing evidence-based approaches and high-quality scientific data.

Additional Elements of a Safety Assessment

Elements of the safety assessments of infant formulas need to be standardized (e.g., toxicity studies, risk assessment). A scientifically rigorous set of guidelines must also allow for flexibility in their approach since specific safety assessments must be targeted according to the nature of the ingredient.

The committee recommends that bioavailability be specifically addressed in any evaluation of the safety of infant formulas. Other factors that should be considered for safety are: tolerance, allergenicity, impact of gastrointestinal flora, and possible nutrient imbalances (if ratios or cofactors are important). While bioavailability is a factor considered under the proposed infant formula regulations, other factors are also of special importance to infants and should be specifically addressed in any evaluation of the safety of infant formulas.

The committee recommends that the manufacturer implement an appropriate in-market surveillance strategy that is based on findings from preclinical and clinical studies and the potential for harm to infants. Infant formula manufacturers routinely conduct passive surveillance (e.g., contact with health care professionals), but guidelines do not exist to assist manufacturers in determining the appropriate type and length of surveillance, from simple methods (e.g., incoming toll-free calls) to rigorous methods (e.g., clinical follow-up of the original study population).

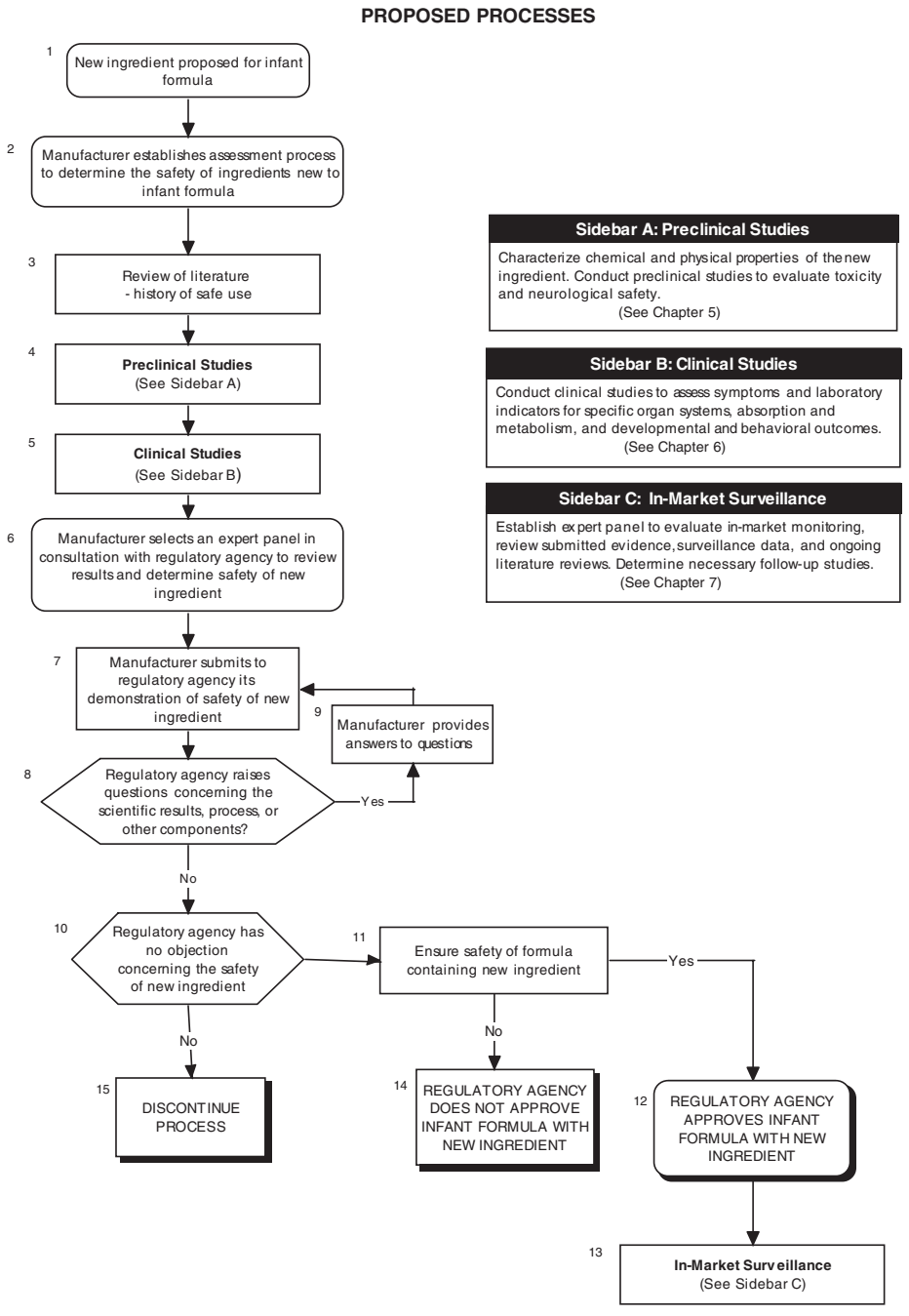

FIGURE ES-1 Proposed process for evaluating the safety of ingredients new to infant formulas algorithm. In-market assessment should be planned in conjunction with preclinical and clinical testing. This algorithm is modeled after the U.S. Generally Recognized as Safe Notification process; similar schemes can be adapted to other regulatory processes. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

Preclinical Studies for Evaluating Safety

Preclinical studies are a vital first step to assess the safety and quality of ingredients new to infant formulas. Guidelines and regulations should consider the special needs and vulnerabilities of infants. Also, the diversity in the ingredients and issues requires clear but flexible preclinical guidelines. For example, a complex mixture or ingredients derived from novel sources or processes present unique safety issues. Also, guidelines should provide the most appropriate animal models at relevant developmental stages for testing infant formulas. For example, the most commonly used animal models for general toxicological studies are the rat and mouse, which are of limited use for developmental studies because of the difficulty of feeding formula to a preweanling rodent. The nonhuman primate and the piglet are more amenable for these types of studies because they readily accept infant formula as a nutrient source.

Current guidelines, provided in the FDA Redbook, and regulations do not address the special needs and vulnerabilities of infants or the diversity of potential ingredients.

The committee recommends the use of a two-level assessment approach to determine the potential toxicity of the ingredient, its metabolites, and their effects in the matrix on developing organ systems (see Figure ES-3, Sidebars A and B). Level 1 assessments include standard measures for each organ system (gastrointestinal, blood, kidney, immune, endocrine, and brain). Level 2 assessments include in-depth measures of organ systems that would be used to explicate equivocal level 1 findings or specific theoretical concerns not typically addressed by level 1 tests. Preclinical assessments range from cellular-molecular through whole animal studies (see Figure ES-2, Sidebars A and B).

The committee recommends that a distinct set of procedures that use an appropriate number and type of animal model at relevant developmental stages be included in preclinical studies. At least two animal models should be selected, and justification for the studies, as well as limitations in extrapolating to humans, should be clear, especially regarding the comparative development between the animal model and the human. The appropriate species, age, and safety factors, as well as other factors, such as the timing, bioavailability of the ingredient, and differences in pharmacokinetics and dietary imbalances, should also be considered.

Clinical Tests for Evaluating Safety

Human clinical studies in infants are a vital second step in the safety assessment process after preclinical studies have been satisfactorily completed. Clinical studies can predict how a new infant formula may affect growth and development, organ systems, and developmental-behavioral outcomes. Clinical studies in human infants are needed for several reasons. First, extrapolation from animal studies may be limited by differences between animal and human structure, physiology, and development. Second, extrapolation from isolated tissue studies is limited by the inability of such models to assess functions in the context of whole organ systems where coordination and integration are the rule. Third, there may be no available animal or tissue model to test specific conditions or functions (e.g., tests for allergenicity or higher cognitive functions found only in humans).

There are no explicit requirements for clinical testing of ingredients new to infant formula specified under Canadian or U.S. regulations. However FDA recommends that the studies conform to guidelines presented in section VI.A. of the Redbook. In addition, in the proposed rule under Section 412 of the FD&C Act, FDA proposes that researchers use the 120-day growth study as the main method to assess the ability of an infant formula to sustain normal infant growth.

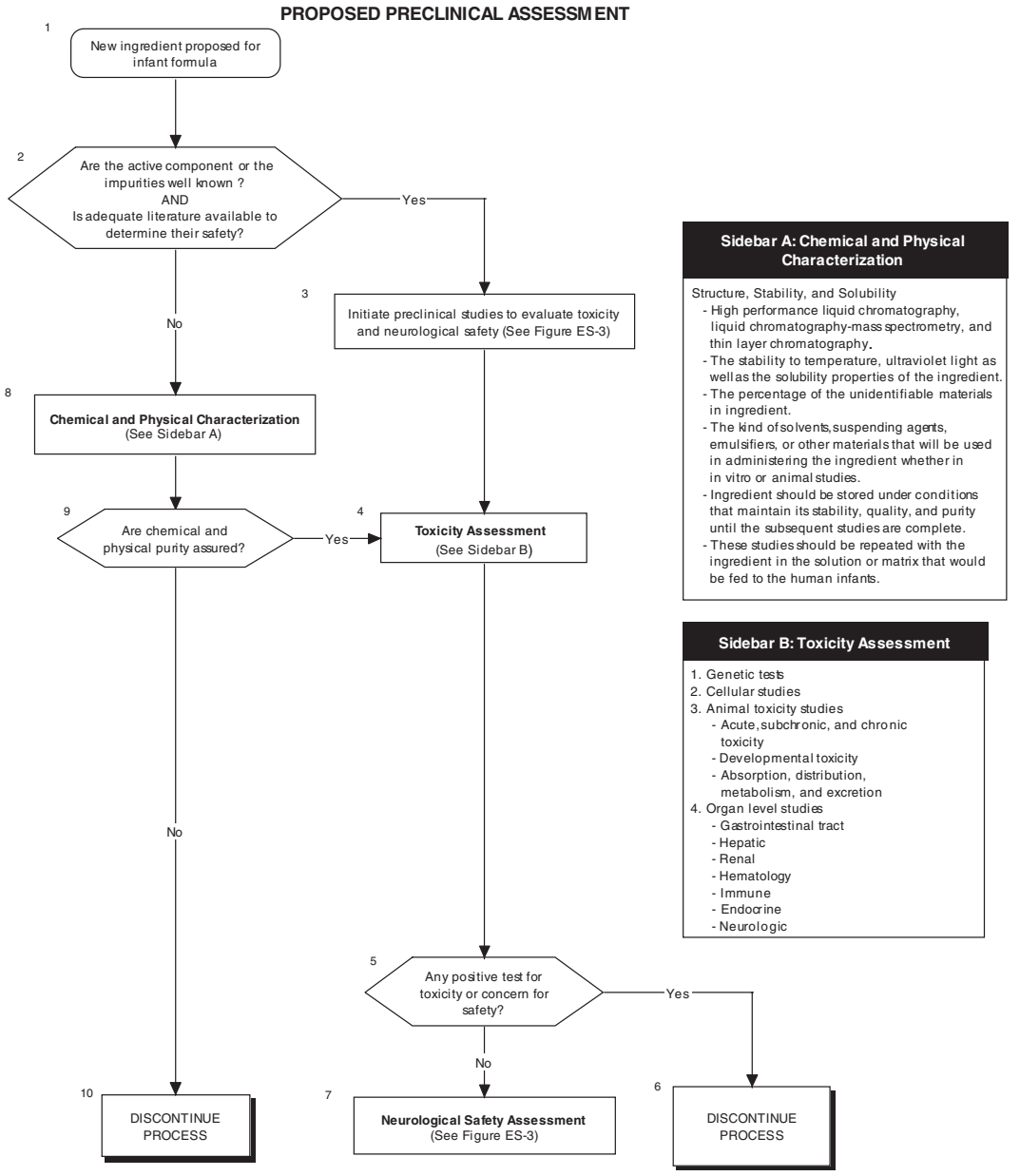

FIGURE ES-2 Proposed preclinical studies algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

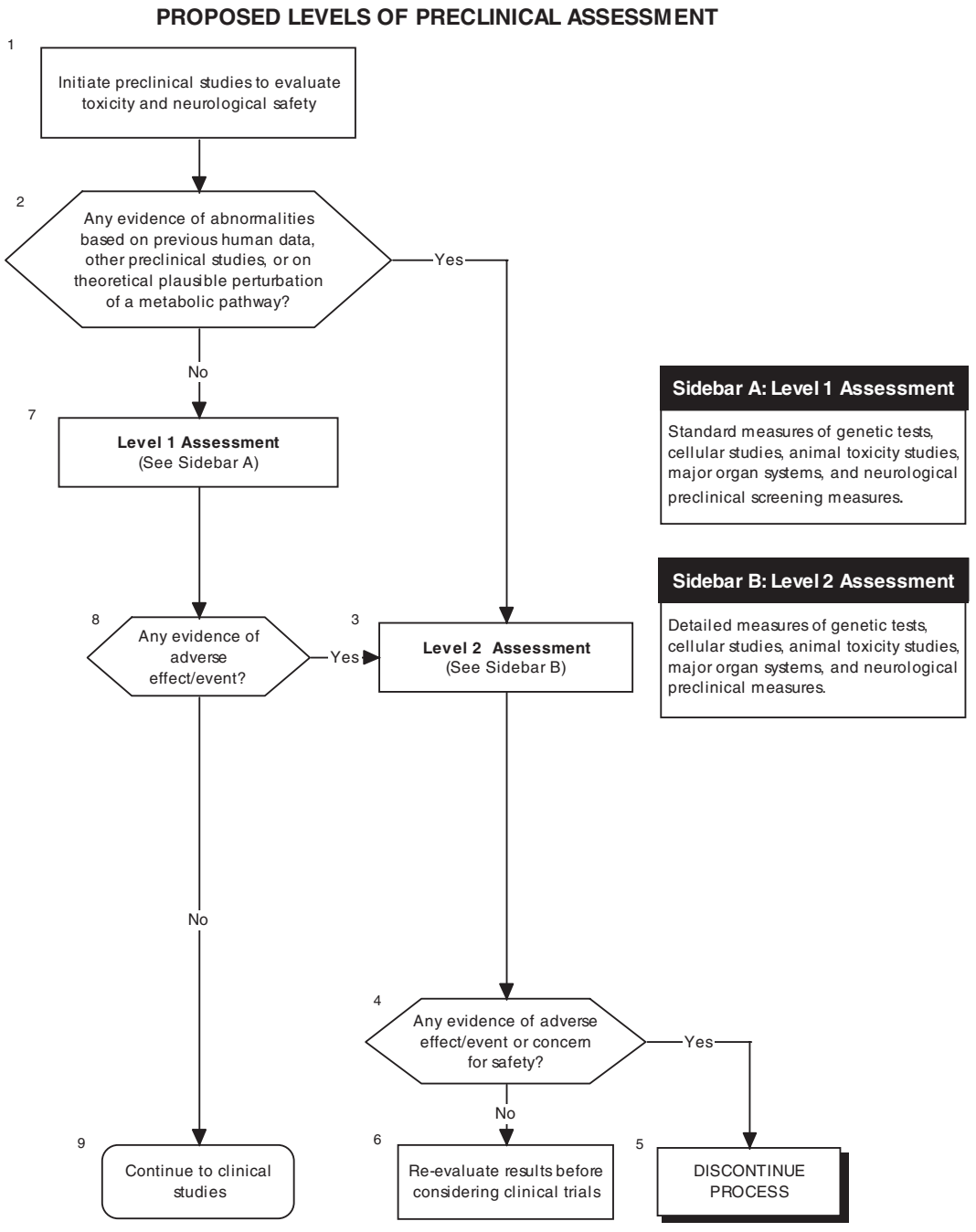

FIGURE ES-3 Proposed levels of assessment for preclinical studies algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

Growth

The committee recommends that growth studies should continue to be a centerpiece of clinical evaluation of infant formulas and should include precise and reliable measurements of weight and length velocity, and head circumference. Appropriate measures of body composition should also be assessed (see Figure ES-4, Sidebar A). These measures help researchers understand the impact of an ingredient new to infant formulas. For example, weight is responsive to acute insults, such as infectious morbidity or changes in nutrient intakes, and recumbent length is an overall indicator of linear or bone growth.

The committee recommends that clinical growth studies follow the study participants for the entire period when infant formula remains a substantial source of nutrients in the diet of the infant. The committee believes that a 120-day growth study, in the 1996 FDA proposed rule, may be insufficient for several reasons. Currently human milk is recommended as the sole nutrient source for infants ages 4 to 6 months; infant formula, intended as a human milk substitute, is recommended for the same period of time. Ideally formula should be tested for the entire period for which it is intended to be fed as the sole source of infant nutrition (up to 6 months, or roughly 180 days, consistent with breastfeeding guidelines) rather than the currently proposed 120-day period. An additional and more serious limitation of the 120-day growth study is that it does not allow for the determination of delayed effects or for understanding longer-term effects of early perturbations in growth.

The committee recommends the development of specific guidelines that define normal growth and establish a level of difference that represents a safety concern. Specifically, the committee recommends that any addition of an ingredient new to infant formulas should be judged against two controls: the previous iteration of the formula without the added ingredient and human milk. The proposed rule does not define "normal" growth, nor does it identify what represents a biologically meaningful difference among groups of infants consuming different formulas. The committee recognizes that there is very little scientific evidence to establish a level of difference associated with long- or short-term health consequences. However the committee concluded that any systematic and statistically significant difference in size or growth rate among infants fed a formula with the new ingredient versus human milk or an already approved formula should be a safety concern.

Organ Systems, Neurobiological Development, and Behavior

In addition to growth studies, the committee recommends a two-level assessment approach to assess organ, immunological, and endocrinological systems (see Figure ES-5, Sidebars A and B). Level 1 assessments include standard measures for each organ system (gastrointestinal, blood, kidney, immune, endocrine). Level 2 assessments include in-depth measures of organ systems that would be used to explicate equivocal level 1 findings or specific theoretical concerns not typically addressed by level 1 tests.

These major organ systems must be studied because growth deficits are likely to appear only secondary to effects on specific organs or tissues and may not appear for some time after nutritional insult. For example, slow or inadequate growth is a common denominator of impaired gastrointestinal function, but it does not identify the function that is impaired. In addition, some organ systems (e.g., immune and endocrine) are immature at birth; every effort must be made to ensure that ingredients new to infant formulas will not affect the development of these systems or the expression of their function.

The committee recommends a three-level assessment approach to assess developmental-behavioral outcomes, including sensory-motor, cognitive development, temperament, and

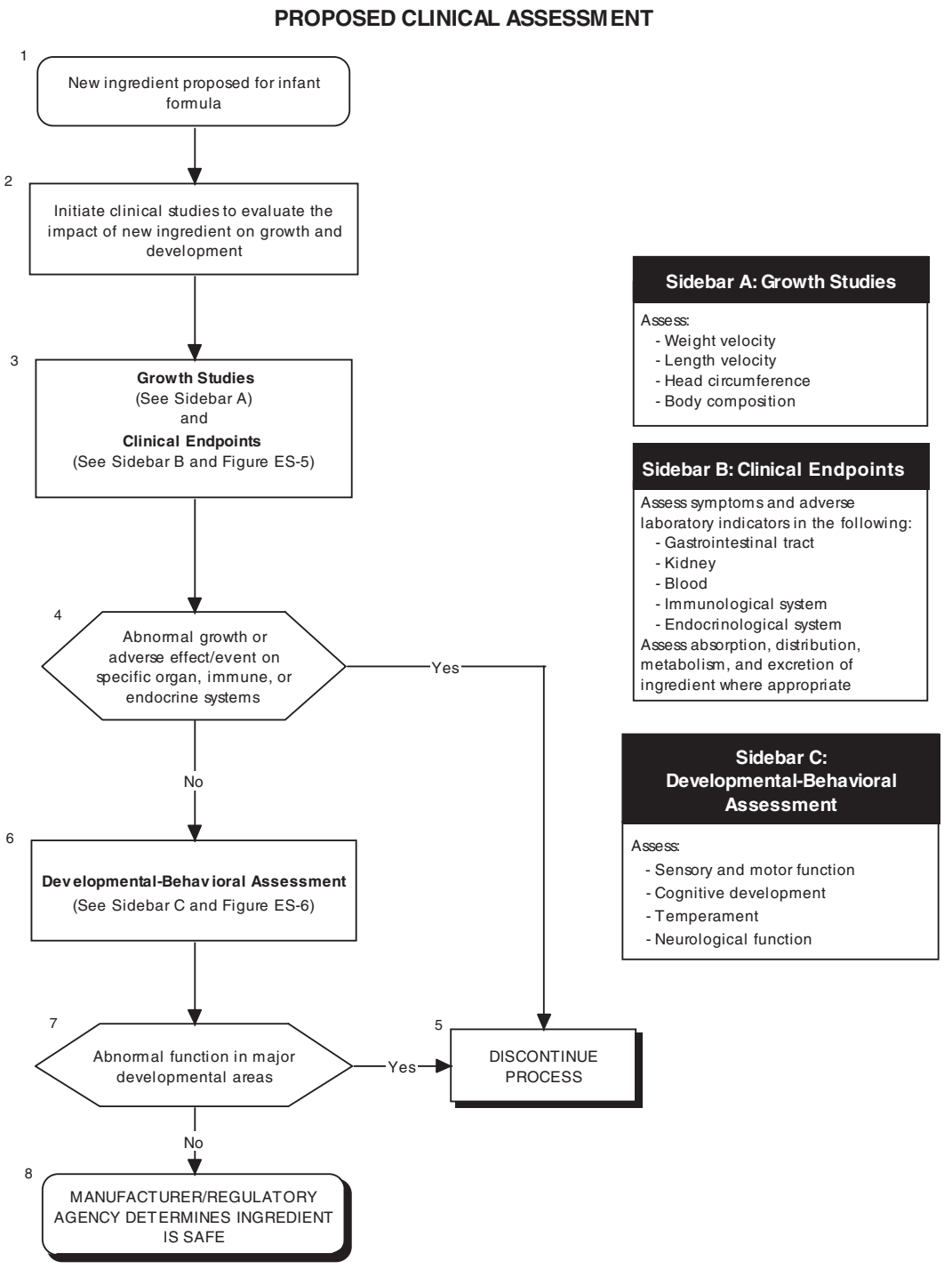

FIGURE ES-4 Proposed clinical studies algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

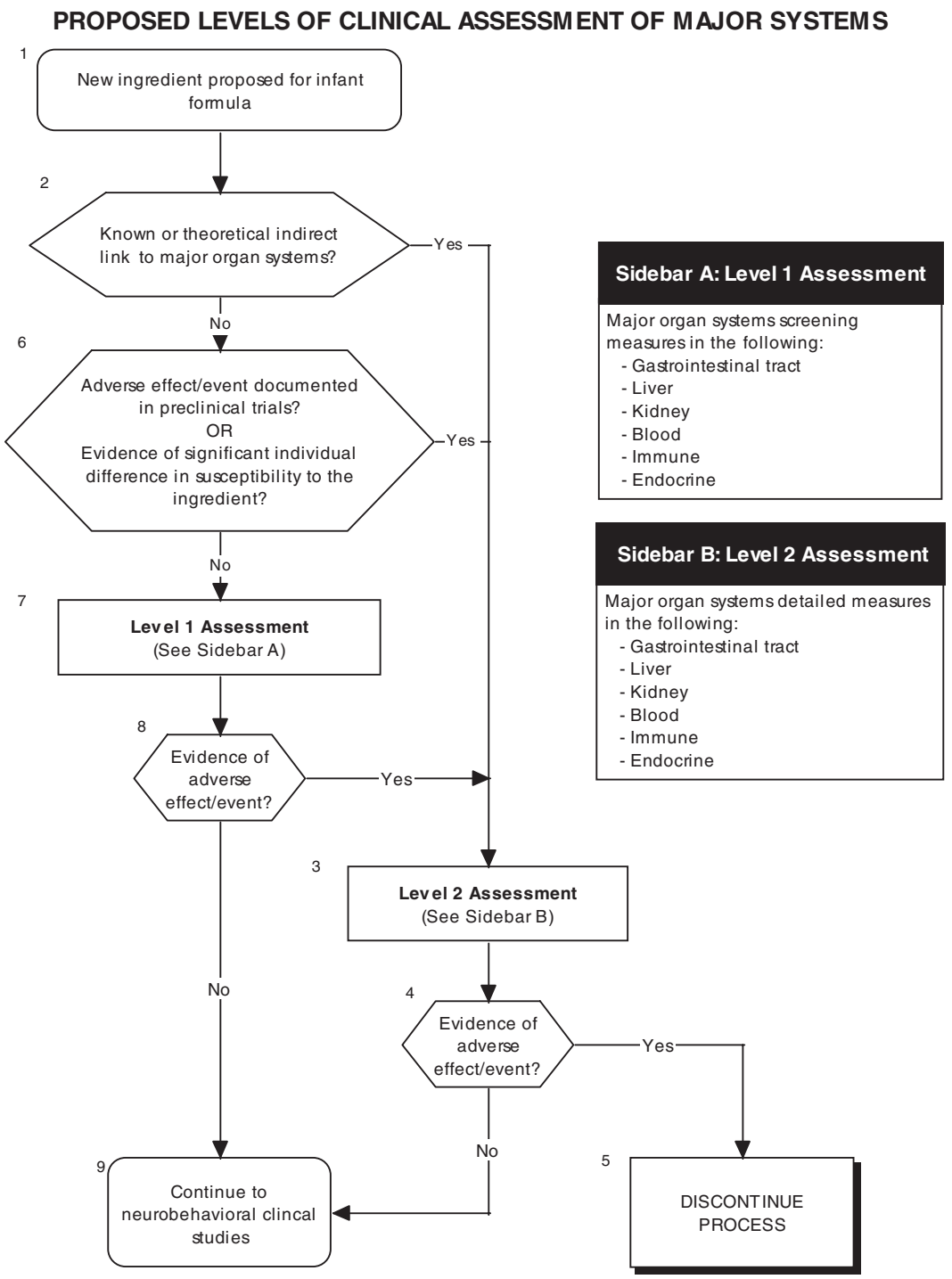

FIGURE ES-5 Proposed levels of assessment for clinical studies of major organ, immune, and endocrine systems algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

neural functions, with appropriate measurement instruments and study design features (see Figure ES-6, Sidebars B, C, and D). Level 1 assessments include developmental screening measures. Level 2 assessments include in-depth measures of child functions in major developmental areas (single assessment with one instrument). Level 3 assessments include in-depth measures using repeated assessments with multiple instruments.

These developmental-behavioral outcomes are important for the following reasons:

- Behavioral outcome measures are sensitive to exposure to toxic substances.
- Developmental-behavioral measures can have long-term predictive value.
- Bidirectional brain-behavior links exist (e.g., brain development mediates changes in behavioral competence, but the child's interactions with his or her environment also can influence brain development).

The proper application of developmental-behavioral studies requires the use of the most appropriate measurement instruments and study design features to assess sensory and motor functions, cognitive development, infant temperament, and neurological function.

In-Market Surveillance to Detect Adverse Effects

Although satisfactory completion of the appropriate preclinical and clinical studies diminishes the likelihood of systematic adverse reactions, the risks for adverse reactions cannot be ignored. Adverse effects may not be detected in preclinical studies if the wrong animal model was chosen, if the assessment instrument chosen measured a function other than the one adversely affected by the new ingredient, or if a subpopulation of individuals who are highly sensitive to the new ingredient added to infant formulas was not sufficiently represented in clinical studies. Also, brain areas that are adversely affected by a new ingredient may not become functionally apparent until later in development. Therefore in-market surveillance is needed to ensure safety and normal development of the infant population.

The committee recommends that all submissions seeking to add a new ingredient to infant formula include a systematic plan for continued in-market monitoring and long-term surveillance (see Figure ES-7). Formal regulatory guidelines for in-market surveillance do not exist for infant formulas. Surveillance is generally limited to consumer reporting of adverse events through toll-free numbers or Internet sites established by the manufacturer or the regulatory agency. There are a number of reasons why this approach is inadequate. One reason is the risk of underestimating actual negative occurrences given that not all caregivers will report a problem. Furthermore, caretakers will be less likely to link a child's problems to earlier intake of infant formula.

The committee, however, recognizes that there are methodological factors (e.g., length of follow-up, confounding factors, lack of statistical power), practical concerns (e.g., continuity of the research team, tracking of subjects, record retention), and cost considerations that limit the implementation of certain in-market surveillance programs.

The committee recommends a three-level assessment approach to determine appropriate in-market surveillance strategies. Level 1 assessments include monitoring the toll-free line or Internet website (passive surveillance). Level 2 assessments include in-market panels to review existing data (both published and proprietary). The same selection and composition recommendations presented earlier also hold with regard to in-market panels. Level 3 assessments include conducting retrospective and/or follow-up studies (active surveillance).

The committee recommends that an expert panel determine the level and strategies to be utilized based on conditions under which potential adverse effects of a new ingredient added

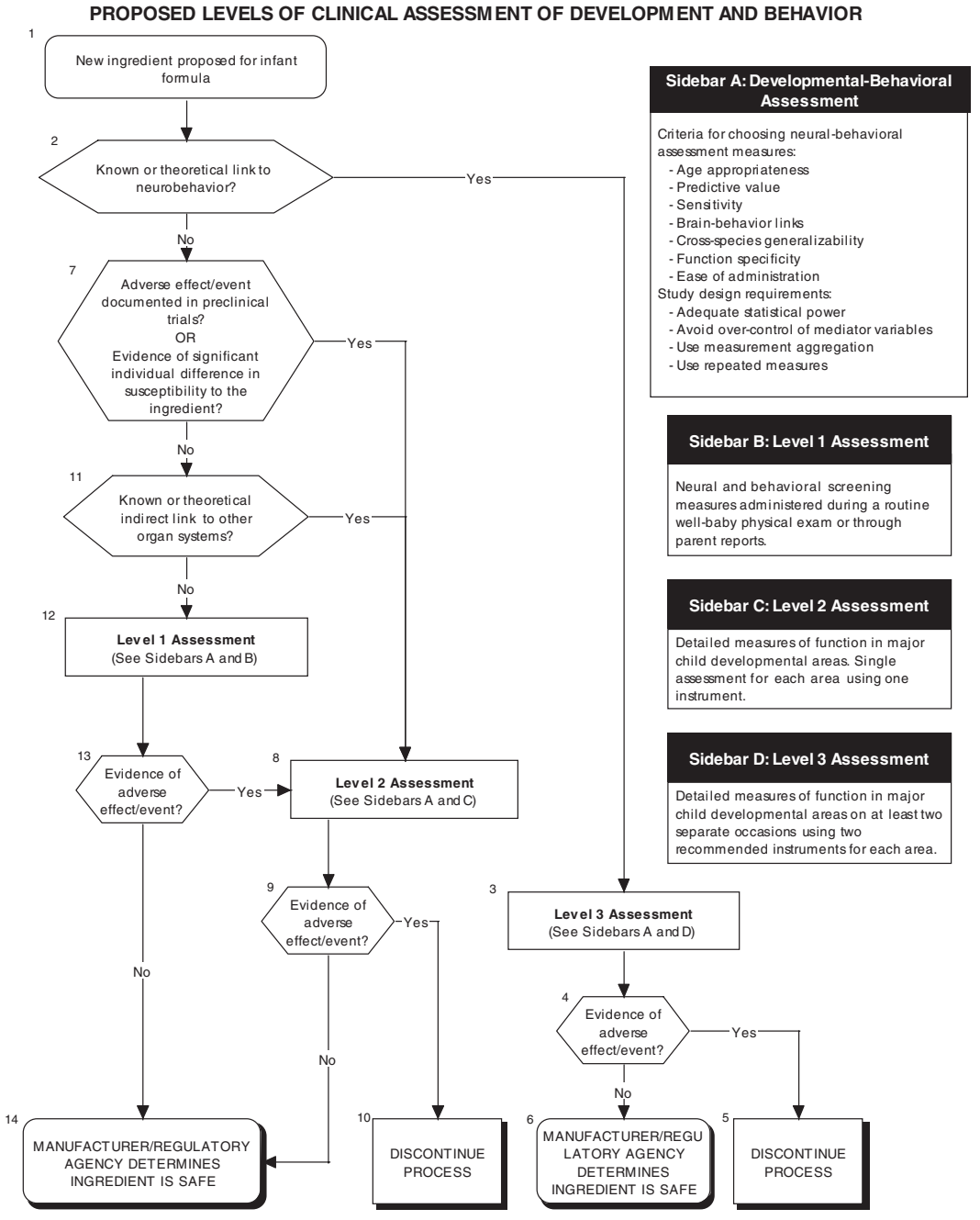

FIGURE ES-6 Proposed levels of assessment for clinical studies of development and behavior algorithm. [ ] = a state or condition, < > = a decision point, [ ] = an action, sidebar = an elaboration of recommendations or statement.

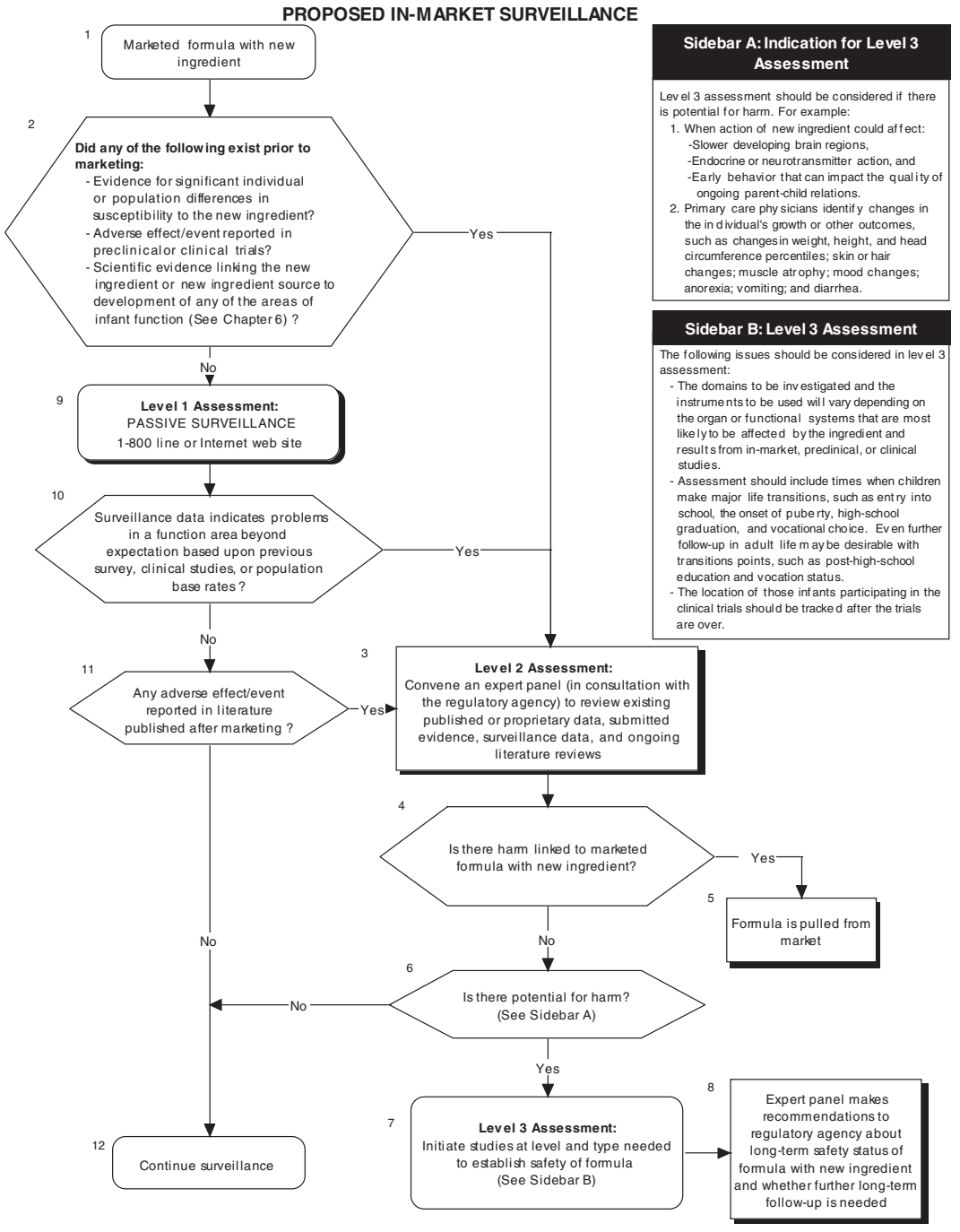

FIGURE ES-7 Proposed in-market surveillance algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

to infant formulas might have been missed in preclinical or clinical trials involving the ingredient.

In-market monitoring information must be assessed for each area of function reviewed, as appropriate. Level 1 assessments are recommended only when *all* the following conditions occur:

- There is no evidence of adverse effects in preclinical or clinical studies, including adverse effects with potentially plausible alternative explanations (e.g., the effects are viewed as the result of random chance or the reviewers believe that there may be methodological or statistical problems in the studies).
- A review of the relevant scientific literature indicates there is no link between the new ingredient, metabolites, secondary effectors, or source and development of any of the areas of infant function previously described.

Level 2 assessments are required when any one of the above conditions does not occur or when level 1 assessments indicate unexpected problems in a function area, based on previous surveys, clinical information, or population-based rates.

In-market follow-up information must be assessed for each area of function reviewed, as appropriate. Level 2 assessments are recommended when *any one* of the following conditions occurs:

- A review of the relevant scientific literature indicates that there is existing evidence linking the new ingredient, metabolites, secondary effectors, or source to the growth and development of organ systems that could result in cumulative adverse effects over time.
- There is evidence of adverse effects in preclinical or clinical studies, including adverse effects with potentially plausible alternative explanations.
- In-market monitoring reveals any adverse effects reported for the new ingredient, metabolites, secondary effectors, or source.

Level 3 assessments for in-market follow-up are required when *any one* of the above conditions occurs and level 2 assessments of in-market follow-up (review by the expert panel) indicate potential for harm in a function area.

CONCLUDING REMARKS

This report describes the critical need to ensure the safety of infant formulas resulting from a number of converging issues:

- Infancy is a uniquely vulnerable period of life.
- Infant formulas are consumed by the vast majority of infants and are the *sole* source of nutrition for a large segment of infants up to the first 6 months of life.
- Manufacturers are increasingly interested in adding new ingredients to formulas in an attempt to mimic the perceived and potential benefits of human milk.
- Existing guidelines and safety regulations lack clarity and completeness in adequately addressing the unique growth and development requirements of infants and the vast diversity of potential new ingredients.

The committee is confident that this report will provide regulatory agencies—FDA, Health Canada, and others—with the recommendations, tools, and resources required to improve guidelines to ensure the safety of infant formulas for generations to come.

1

Introduction and Background

Infant formulas are liquids or reconstituted powders fed to infants and young children to serve as substitutes for human milk. Infant formulas have a special role in the diet because they are the only source of nutrients for some infants. In the United States and other industrialized countries, the vast majority of infants receive infant formula at some time during their first year of life (Hediger et al., 2000; Ryan et al., 2002) as the number of infants breastfed after birth rapidly decreases (Figure 1-1). Many infants receive formula in combination with breastfeeding. During these mixed feeding routines there are potential interactions between the components of human milk and those contained in formulas.

Over recent decades ingredients have been added to infant formulas not only to better simulate the composition of human milk, but also to impart health benefits. Examples include fortifying formulas with iron, adding nucleotides, and changing the composition of fat blends. Recently infant formulas containing added sources of arachidonic acid and docosahexenoic acid have been made available in the United States, Europe, and elsewhere. In the United States, new ingredients, such as probiotics and compounds produced by genetic engineering, are currently being considered for addition to formulas.

Infancy is a uniquely vulnerable period of rapid growth and development and as such, feeding changes have the potential to impart benefit or harm in the short term, into early childhood, and even later into adulthood. Thus measurements of safety parameters during infancy need to be equally or even more stringent than at other periods during the life cycle. The introduction of new ingredients to formulas must pose no or minimal risk to infants. In the United States and worldwide there is a paucity of guidelines or recommendations from national and international organizations regarding approaches to assess the safety of ingredients added to infant formulas.

The Committee on the Evaluation of the Addition of Ingredients New to Infant Formula was convened by the Institute of Medicine at the request of the Food and Drug Administration (FDA) and Health Canada to review methods currently used to assess new ingredients to be added to infant formulas, including preclinical and clinical studies and in-market monitoring, and to identify gaps in current safety regulations and guidelines. The committee

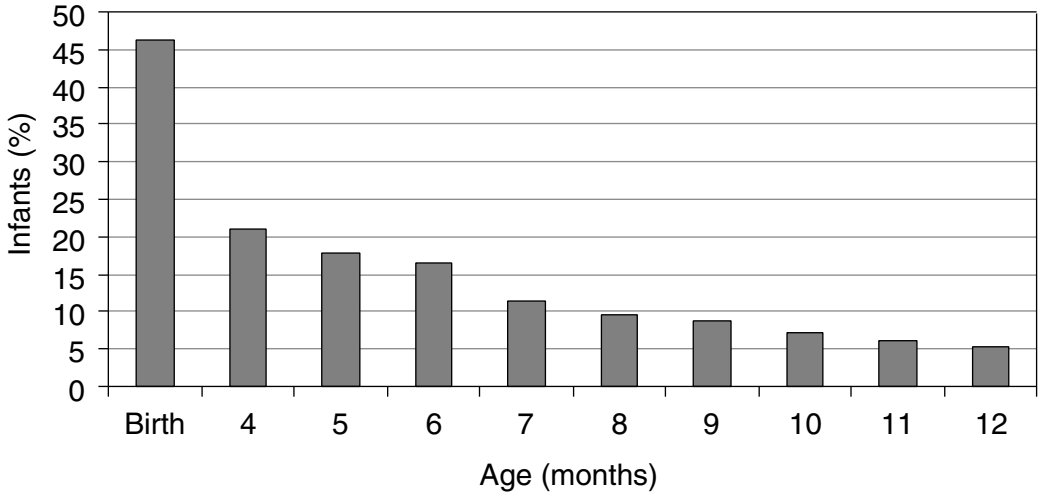

FIGURE 1-1 Percent of infants who were exclusively breastfed from birth to 12 months of age in the United States.
SOURCE: Adapted from Hediger et al. (2000) and Ryan et al. (2002).

met six times over 18 months to fulfill its charge. This report is intended to provide recommendations for regulatory bodies, industry, and basic and clinical investigators involved in determining the safety of new ingredients added to infant formulas.

INFANT FORMULA REGULATIONS AND GUIDELINES

United States

Food products that are designed and marketed for infants are regulated under the Federal Food, Drug and Cosmetic (FD&C) Act of 1938 (21 U.S.C. §301) (Vanderveen, 1991). FDA, an agency in the U.S. Department of Health and Human Services (HHS), regulates infant formulas and evaluates the safety of food and color additives.

Two sections of the FD&C Act, 409 and 412, are the primary laws that relate to infant formulas. Section 409 gives authority to HHS to ensure the *safety* of new food ingredients (e.g., food additives and Generally Recognized as Safe [GRAS] substances) "under the conditions of its intended use" (e.g., in infant formulas). Manufacturers may propose the addition of new ingredients to infant formulas in the United States by either filing a Food Additive Petition with FDA to request a formal premarket review, or making a GRAS determination. It is important to point out that it is the *use* of the substance, rather than the substance itself, that is eligible for the GRAS determination (see Chapter 4).

Under Section 412 of the FD&C Act, regulations have been promulgated and ultimately implemented that are intended to ensure proper formulation for infants to thrive. They include:

- Infant Formula Quality Control Procedures (21 C.F.R. §106),
- Records and Reports Regulations (21 C.F.R. §106.100),
- Infant Formula Labeling Requirements (21 C.F.R. §107.10–107.30),

- Exempt Infant Formulas (21 C.F.R. §107.50),
- Nutrient Requirements for Infant Formulas (21 C.F.R. §107.100), and
- Infant Formula Recall Requirements (21 C.F.R. §107.200–107.280).

FDA (1996) has proposed to revise these regulations to establish quality factors, current good manufacturing practices, and revised quality control procedures. Table 1-1 lists some of the U.S., Canadian, and European Union laws and regulations related to adding new ingredients to infant formulas.

Other Countries

Canada

The Canadian Food and Drug Regulations (Canada, 2001) include specific requirements for infant formulas, novel foods,[1] and other ingredients. Division 25 of the Regulations provides for the addition to infant formulas of nutritive substances, in addition to specified vitamins and mineral nutrients, found in human milk, provided the nutritive substance is added to the formulas to the level found in human milk (section B.25.056). The Regulations include an inclusive list of those food additives that may be added to infant formulas (section B.25.062). Similar to the process in the United States, a new food additive must undergo premarket approval. The manufacturer must submit a request to the Minister of Health that includes all the details laid out in Division 16 of the Regulations. The request must include "detailed reports of tests made to establish the safety of the food additive under the conditions of use recommended" and "data establishing that the food additive will have the intended physical or other technical effect" (section B.16.002). Health Canada reviews the request and, if accepted, the Minister of Health recommends to the Governor-in-Council that the ingredient be added to the list of food additives in the Food and Drug Regulations. The Food and Drug Regulations also require a premarket notification for novel foods. The manufacturer of a novel food must submit a premarket notification for the food as required under Division 28 of the Regulations. Health Canada issues a written notice to the manufacturer if it is satisfied that information submitted establishes that the novel food is safe for consumption.

European Union

The European Union also has regulations in place for food additives, novel foods, and genetically modified organisms. However the European regulations are not specific for adding new ingredients to infant formulas.

[1]As defined by the Food and Drug Regulations, a novel food is "a substance, including a microorganism, that does not have a history of safe use as a food; a food that has been manufactured, prepared, preserved or packaged by a process that (i) has not been previously applied to that food, and (ii) causes the food to undergo a major change; and a food that is derived from a plant, animal or microorganism that has been genetically modified (i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism, (ii) the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism, or (iii) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism" (Canada, 2001, B.28.001).

TABLE 1-1 Selected Laws and Regulations Related to New Ingredients Added to Infant Formulas

Country or Organization	Regulations (Reference)	Identifying Terms
United States	Federal Food, Drug, and Cosmetic Act (21 U.S.C. §301)	Food additive, Substances Generally Recognized as Safe (GRAS)
	Food Additive (21 C.F.R. §170, 171, and 172)	Food Additive
	GRAS (21 C.F.R. §182, 184, and 186)	GRAS
Canada	Food and Drugs Regulations (Canada, 2001)	Food additive, novel food
European Union	European Communities (Infant Formulae and Follow-on Formulae) Regulations, 1998 (FSAI, 1999)	Established by generally accepted scientific data
	Council Directive 89/107/EEC (EEC, 1989)	Food additive
	European Parliament and Council Directive 94/34/EC (EEC, 1994)	Food additive
	Regulation EC No. 258/97 (EC, 1997)	Novel foods, novel food ingredients
	Council Directive 90/220/EEC (EEC, 1990)	Genetically modified organisms

In June 2002 representatives of academia, the infant food industry, the European Commission, food regulatory bodies of some European Union member states, and consumer organizations met to discuss opportunities to evaluate the safety of ingredients new to infant formulas (Koletzko et al., 2002). Topics included consumer expectations, preclinical and clinical evaluations, investigations of infant growth and nutrient bioavailability, and in-market surveillance. The participants also discussed a position paper written by members of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition's Committee on Nutrition (Aggett et al., 2001). The paper provided 13 points for consideration for the evaluation of infant formulas; these points are presented in Box 1-1.

CHARGE TO THE COMMITTEE

FDA and Health Canada asked the committee to review methods currently used to assess new ingredients to be added to infant formulas, including preclinical and clinical studies and in-market monitoring, and to identify gaps in current safety regulations and guidelines. As part of its task, the committee was requested to provide recommendations to strengthen the scientific approaches used in assessing the safety of ingredients added to infant formulas. The committee was asked to focus on:

- ingredients new to infant formulas that are *regulated under the food provisions of the law*, not as drugs or therapeutic agents;
- the health and well-being of *healthy term infants* (delivered between gestational age of 37 to 42 weeks with birth weights of 2.5 kg or more) from birth to 12 months of age; and
- the effects that ingredients new to infant formulas could have on metabolism, physiology, neurological function, and normal growth and development, as well as on specific systems, including the immunological, renal, hepatic, hematological, and gastrointestinal systems.

The committee was asked to address the composition of human milk and to evaluate how the presence or absence of a substance in human milk may be factored into the safety

BOX 1-1 Key Points of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition's Committee on Nutrition Commentary on the Nutritional and Safety Assessment of Breast Milk Substitutes and Other Dairy Products for Infants

1. Although human milk composition can be a guide to that of breast milk substitutes, the comparison of outcomes with those seen in healthy infants who have been exclusively breast fed for 4–6 months is considered a better approach.

2. Appropriate clinical studies of nutritional and safety assessment should be performed particularly for components, and combinations of components, which have not been previously included in infant formulas and other dietary products for infants. Technological as well as compositional modifications to infant formulas should be assessed nutritionally.

3. The introduction of any modification to a formula or other dietary product for infants should be based on a systematic review of the relevant existing information to develop a clear hypothesis of the expected functional and clinical benefits. These reviews should be published or be made publicly available in other forms. Studies should be designed primarily to test these hypotheses, as well as making general nutritional assessments.

4. Infant formulas or other products modified for reasons other than to provide a novel functional or clinical benefit, or which are based on products already on the market, should, at least, be subjected to studies of acceptability, and of nutritional equivalence to the existing products.

5. All infants in clinical trials should be characterized with regard to factors which might affect the planned outcomes. Blind randomization with respect to the allocation of test and reference formulas is important, and all studies should comply with Good Clinical and Good Laboratory Practices.

6. For all clinical trials on nutritional products, ethical approval should be acquired, informed parental consent obtained and this should be declared in the publication of results.

7. Modifications of infant formulas and other dietetic products for infants need to be evaluated for their safety. It is important that the possibility of unexpected adverse outcomes be addressed by adequate clinical monitoring of participants, and by incorporating, into the study design, arrangements for the independent scrutiny of the accumulating data.

8. The general principles, design, execution and the data analysis of evaluative studies of infant formulas and other substitutes for breast milk need to be determined to detect relevant short- and long-term (i.e., in later childhood and adult life) outcomes. The design should consider from the outset the statistical power of the study, and the confidence limits of any differences found should be included in the published reports.

9. Preliminary pilot studies of the proposed study design are often useful to identify and anticipate outcomes and issues which would inform definitive studies and enable protocols to be adapted and would enable the views of the infants' carers to be taken into account. This approach would be expected to enhance the co-operation of carers and the quality of the methodology of the subsequent definitive assessment.

10. Manufactures and scientific, academic, and professional groups should collaborate to the extent of agreeing on an essential portfolio of data and outcomes, which should be recorded in all nutritional studies performed during early life. This would enable the later consolidation of information from individual studies into larger databases which would be appropriate for the assessment of long-term nutritional efficacy and safety, as well as being able to detect unanticipated outcomes of early feeding practices and dietary exposure.

11. A register of current trials of infant formulas should be established, and wherever possible, this information should be accessible to manufacturers and to clinical researchers. It should be used to reduce overlap between investigations, should avoid unnecessary replication of studies, and encourage collaborative projects particularly in the evaluation of pre-competitive modifications. Similar collaborations would facilitate the creation of cohorts, which should be large enough to enable follow-up of the studied infants through their childhood and into adulthood. It was considered possible to achieve this without compromising intellectual property rights, commercial confidentiality and competition between manufacturers.

(continued on next page)

BOX 1-1 *(continued)*

12. Original study records, with protection of the participants' confidentiality, should be preserved wherever possible; from these an anonymous data archive could be made publicly available for retrospective epidemiological assessment of associations with adverse and beneficial outcomes.

13. The results of studies and nutritional trials in infants that have not been completed or have not been published for other reasons should at least be made available publicly, and to the consolidated database, together with the specific reasons for the cessation of the study. Similarly, the specific reasons why children withdrew from completed studies should be included in the published reports.

SOURCE: Reprinted, with permission, from Aggett et al. (2001).

assessment of that substance for use in infant formulas. The committee was also asked to consider how the process of estimating intakes and safety of substances intended for infant formulas has evolved over time and to discuss whether and how this process is changing in light of the current state of clinical science to safeguard the health and well-being of infants enrolled in clinical studies. In addition, the committee was requested to apply its recommended approaches to the specific situation of adding long-chain polyunsaturated fatty acids (LC-PUFAs) to infant formulas for use in term infants. The approaches were to also be applied to other ingredients, if appropriate.

The committee's primary charge was to provide guidelines for assessing the safety of new ingredients added to infant formulas under U.S. regulations, with possible international applications. Under U.S. regulations, the process that evaluates the safety of ingredients is stated in the FD&C Act, but it does not address issues of efficacy (i.e., health benefits). Although the committee recognizes that efficacy should be considered when assessing new ingredients to be added to infant formulas, efficacy is not a consideration under current or proposed regulations for infant formulas or infant formula ingredients. The committee therefore focused its attention on matters related to safety as delineated in its charge. Similarly, although the committee recognizes that cost can be a factor for expert panels making decisions about the appropriate studies to be used to assess safety, cost issues were not included in the charge and, consequently, were not part of its deliberations.

The committee recognizes that some of its recommendations could not be implemented under the current U.S. laws and may require statutory changes. Even with this limitation, the committee encourages dialogue among members of government agencies, the public, industry, and academia to act on the recommendations set forth by this report in the best interest of our most vulnerable members of society—our infants.

THE COMMITTEE'S APPROACH

Types of Ingredients

The committee focused on new potential ingredients and existing ingredient ratios that could be added to infant formulas to:

- impart potential health benefits within the first year of life, later in childhood, and perhaps even during adult life; and
- change color, extend shelf life, or modify marketing or manufacturing processes because they could potentially be associated with harmful short- or long-term effects.

Several compounds have been seriously considered for addition to formulas, but no final decision has been made about their addition for a variety of reasons. For example, cholesterol is present in human milk at levels higher than is present in cow's milk. A recent article reported that breastfed infants had lower total cholesterol and low-density lipoprotein cholesterol in adulthood and suggested that infant formulas should have added cholesterol to more closely match that of human milk (Owen et al., 2002). On the other hand, animal studies of formulas with added cholesterol found no evidence of any beneficial short- or long-term effects (LSRO, 1998). In addition, lysozyme and lactoferrin are also present in human milk at levels higher than in cow's milk. These factors may be important in growth or host defense and have been considered for addition to formulas (Lo and Kleinman, 1996). Many substances found in human milk have not been added to infant formulas, perhaps due to the lack of information on their function in human milk or their effect on the infant.

The committee proposes a functional classification of potential target areas of ingredients new to infant formulas, as summarized in Table 1-2, according to potential targeted endpoints. The committee also considered the types of molecules or ingredients that could be considered as new additives, including:

- new sources of existing ingredients;
- prebiotics and probiotics;
- lipids;
- nitrogen-containing ingredients (e.g., recombinant proteins; single amino acids, such as glutamine, and immunoglobulins; and bioactive peptides/polyamines/proteins, such as lactoferrin, enzymes, and hormones);
- oligosaccharides;
- vitamins, minerals, and flavonoids;
- flavoring and coloring agents; and
- nonprotein recombinant-derived products.

However the committee did not simply consider molecules or new ingredients as isolated substances, it also considered three important characteristics: (1) the compound itself

TABLE 1-2 Functional Classification of Potential Target Areas of New Ingredients

Class	Examples	Examples of Possible Adverse Consequences
Anti-allergic	Prebiotics, probiotics, cytokines	Gastrointestinal side effects
Anti-infective	Antiviral/bacterial agents, probiotics, prebiotics, lactoferrin	Adverse immunological changes, allergic effects
Flavor/color/texture/ stabilizing agents	Organoleptics, flavors, aeromatics	Toxicity, carcinogenicity
Immunologic	Long-chain polyunsaturated fatty acids, enzymes, cytokines, nucleotides	Immunological, inflammatory effects
Metabolic	Enzymes, hormones	Allergic immunological changes, allergic effects
Neurodevelopmental and behavioral	Long-chain polyunsaturated fatty acids, growth factors, choline, oligosaccharides, precursors of neurotransmitters, neurotransmitters, amino acids	Decreased growth, decreased cognitive ability
Trophic	Growth factors, hormones	Abnormal increase or decrease in growth, endocrine effects

(*the molecule*), (2) *the matrix* in which it is delivered, and (3) *its amount and ratio* relative to other constituents in the infant formula. The committee also realized that certain potential new additions to infant formulas might be in the form of complex ingredients or biologicals (e.g., probiotics). Some of the recommended chemical, physical, and in vitro characterization steps might not apply to complex ingredients (see Chapter 5).

The committee also reviewed whether ingredients derived from genetically engineered techniques should have the same safety recommendations for use in infant formulas as compared with other classes of new ingredients. Even though compounds derived from genetic engineering techniques would have properties similar to those of natural compounds, they would still have to be tested. Similar to other ingredients, the short- and long-term unintended compositional changes that result directly or indirectly as a result of genetic engineering would need to be considered. In addition, the unintended functional consequences of a successful, specific intentional alteration would need to be considered and monitored over long time periods. Thus the committee recommends that standards for safety should be the same for ingredients derived from genetically engineered techniques as they are for other classes of ingredients. Guidelines should maintain emphasis on a "reasonable certainty of no harm" for *all* ingredients.

Safety Definitions

The committee reviewed other existing reports and guidelines, including those available from FDA and the Life Sciences Research office (LSRO), and it considered current recommendations relating to safety. While the committee recognized that its recommendations had to be targeted with safety as the ultimate objective, additions of ingredients new to infant formulas need to consider potential efficacy (i.e., health benefits) considerations.

The concept of "safety" refers to a reasonable certainty of no harm per the FD&C Act; to safe and adequate levels of nutrients, including essentiality, stability, history of use, and toxicity per LSRO; or, in some circumstances, a reasonable balance between costs (e.g., risks, harm) and benefits. Safety, therefore, is not an inherent biological property, but rather a point on a continuum that is influenced by intellectual concepts and judgment. Generally, a "hazard" refers to a substance or combination of substances that produce undesired outcomes; in the case of infant formulas, the undesired outcome is health related. "Risk" implies that an adverse event will be expressed under specified conditions, and "harm" refers to the nature of an undesired outcome associated with a hazard. Details about the concept of safety and surrounding issues are provided in Chapter 2.

Use of a Hierarchical Approach to Safety Assessment

The committee recognized that its charge was to provide comprehensive guidelines for evaluating the safety of the addition of ingredients new to infant formulas—not to produce a "how to" document. With this in mind, the committee discussed an initial outline and agreed that for each area it would review the existing systems, identify gaps or limitations of the systems, and make recommendations for revised guidelines. The committee also agreed on the concept of a hierarchical approach to determine levels of safety assessment needed for each ingredient.

To evaluate the safety of the addition of new ingredients to infant formulas, the committee proposes the use of a hierarchical approach, which involves matching the level of safety assessment with the degree of concern about the potential harm. Such an approach needs to

be considered in terms of a new ingredient's potential: (1) harm (e.g., toxicity), and (2) adverse effects. This hierarchical approach will guide the levels of assessment applied depending on the nature and purpose of the new ingredient.

Considerations about the degree of concern and, therefore, about the level of safety assessment required would include: whether potential harmful effects of a new ingredient would be reversible or irreversible, the severity of the effect, and the consequences. For example, levels of safety assessment might not be as intense for an ingredient with a proven lack of adverse effects (e.g., an ingredient added for formula stability) as compared with a new ingredient added to induce a positive biological effect (e.g., better visual acuity). In its hierarchical approach, the committee includes assessments that consider whether an adverse effect manifests immediately (while the infant is ingesting the formula) or later (even into adulthood and future generations). The committee also recommends assessing the likelihood that a new ingredient could adversely affect a specific system and whether the effect would be common or rare.

To help facilitate the hierarchical approach, the committee recommends the use of algorithms. An algorithm diagrams the process into a step-by-step decision tree. The steps in the algorithm include:

- recommended observations and assessments,
- decisions to be considered, and
- actions to be taken.

Standardized symbols are used in a flowchart to display each step in the algorithm (SMDMC, 1992) (Figure 1-2). Arrows connect the numbered boxes, indicating the order in which the steps should be followed. A letter within a box of an algorithm refers the reader to corresponding text (or sidebar). The sidebar elaborates on the recommendations and statements that are found within each box of the algorithm. Readers should keep in mind that the algorithm depicts the logic of the process, but does not denote a chronology (e.g., a manufacturer may initiate several different studies and procedures at the onset of the process, the results of which are assessed at different steps of the algorithm).

The algorithms presented in Chapters 4 through 7 are designed to encompass a broad spectrum of components for evaluating the safety of new components added to infant formulas. The use of algorithms in applying the hierarchical approach provides several advantages. First, the utilization of algorithms should simplify the process of planning the type and depth of safety assessments for each new ingredient. Second, evidence suggests that an algorithm approach improves data collection, problem solving, and decision making. Third, multiple levels of information are incorporated into a single, unified document. Finally, the algorithmic format allows the regulatory agency and the manufacturer to follow a linear approach to critical information needed at the major decision points.

The algorithms in this report are provided as generic guides and as tools for stepwise approaches to be used in assessing the safety of ingredients new to infant formulas. The committee realizes that it cannot provide specific recommendations for each compound and that some variation in these approaches may be needed for specific ingredients. Thus the committee recommends that the manufacturer and an expert review panel establish the relative importance of potential adverse effects for each specific new ingredient and determine the depth of preclinical and clinical studies and in-market surveillance needed to assess safety.

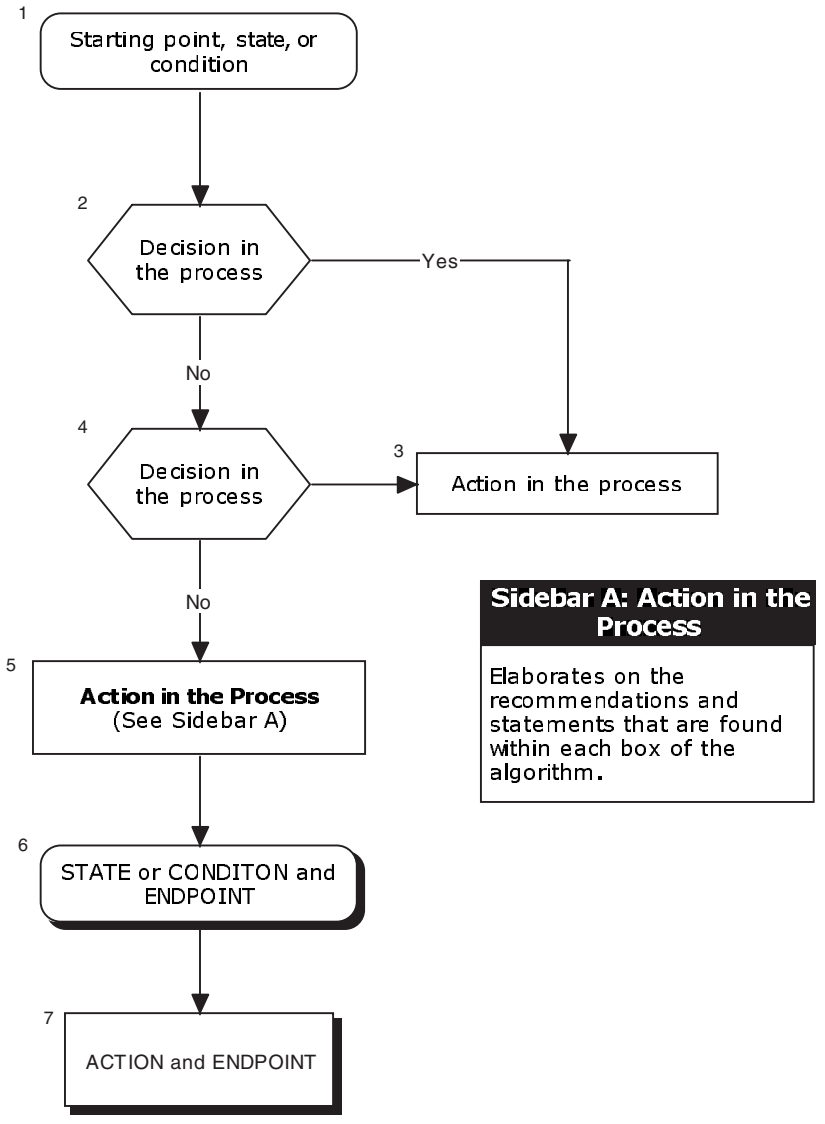

FIGURE 1-2 An example of an algorithm.

Using this flexible, stepwise approach, each potential new ingredient is considered using evidence-based approaches and high-quality scientific data to assess potential adverse effects on:

- growth and development (including temperament),
- the immune system (including allergic and infectious risks),
- metabolism (e.g., acid-base balance, effects related to lipid and carbohydrate processing), and
- other organ systems (e.g., hematological, gastrointestinal).

For each distinct area of safety assessment (i.e., preclinical, clinical, and in-market monitoring) the committee designed algorithms or stepwise decision trees to be applied to new ingredients for infant formulas. The committee's approach was based on the uniqueness of the infant population. Therefore each step in the process requires empirical evidence from many disciplines and the application of the highest standards, whether using methods of bioassay, nutritional analysis, or basic chemistry. This approach could be applied to new ingredients to be added to infant formula regardless of the regulatory process used.

ORGANIZATION OF THE REPORT

The committee structured this report so that the first three chapters provide the background information and rationale for the report. Chapter 2 reviews the parameters considered by the committee when defining "safety" and how to approach it from a practical, theoretical, and statistical point of view. Chapter 3 compares the biological and behavioral advantages of human milk with infant formulas and reviews how infant formulas were developed to meet the biological advantages of human milk.

The remainder of the report reviews the current regulatory processes involved in evaluating the safety of ingredients new to infant formulas and provides recommendations for the overall process (Chapter 4), preclinical studies (Chapter 5), clinical studies (Chapter 6), and finally, in-market surveillance (Chapter 7). In Appendix D the committee provides two case studies that have recently utilized the current regulatory process by submitting a GRAS Notification. The addition of LC-PUFAs and probiotics to infant formulas are used as examples of how the recommendations of the committee could be utilized for new ingredients in infant formula. LC-PUFAs may affect diverse functions within the brain and therefore demonstration of safety is critical; probiotics is presented because it is a complex ingredient that could potentially impact diverse organ systems and could potentially raise allergenicity concerns. The purpose of this exercise was not to make conclusions about the completeness of the information or the safety of the new ingredients, but to point out important assessments or steps that could be considered. In doing so, the committee emphasizes the importance of including some steps, such as follow-up surveillance and level 2 assessments, in future assessments of the safety of the addition of new ingredients to infant formulas.

SUMMARY

The Committee on the Evaluation of the Addition of Ingredients New to Infant Formula provides FDA, Health Canada, and other regulatory bodies worldwide with a thorough discussion of the challenging issues surrounding the determination of the safety of new ingredients to be added to infant formulas. The committee encourages dialogue among members of government agencies, the public, industry, and academia to act on the recommendations set forth by this report in the best interest of our most vulnerable members of society—our infants.

REFERENCES

Aggett PJ, Agostini C, Goulet O, Hernell O, Koletzko B, Lafeber HL, Michaelsen KF, Rigo J, Weaver LT. 2001. The nutritional and safety assessment of breast milk substitutes and other dietary products for infants: A commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 32:256–258.

Canada. 2001. *Departmental Consolidation of the Food and Drugs Act and the Food and Drug Regulations*. Ottawa: Minister of Public Works and Government Services Canada.

EC (European Communities). 1997. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *Off J Eur Communities* L043:1–7.

EEC (European Economic Communities). 1989. Council Directive of 21 December 1988 on the approximation of the laws of the member States concerning food additives authorized for use in food stuffs intended for human consumption (89j/107/EEC). *Off J Eur Communities* L40:27.

EEC. 1990. Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC). *Off J Eur Communities* L117:15.

EEC. 1994. European Parliament and Council Directive 94/34/EC of 30 June 1994 amending Directive 89/107/EEC on the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption. *Off J Eur Communities* L237:1.

FDA (Food and Drug Administration). 1996. Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula: Proposed rule. *Fed Regist* 61:36153–36219.

FSAI (Food Safety Authority of Ireland). 1999. *Recommendations for a National Infant Feeding Policy*. Dublin: FSAI. Pp. 94–121.

Hediger ML, Overpeck MD, Ruan WJ, Troendle JF. 2000. Early infant feeding and growth status of US-born infants and children aged 4–71 mo: Analyses from the Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 72:159–167.

Koletzko B, Ashwell M, Beck B, Bronner A, Mathioudakis B. 2002. Characterisation of infant food modifications in the European Union. *Ann Nutr Metab* 46:231–242.

Lo CW, Kleinman RE. 1996. Infant formula, past and future: Opportunities for improvement. *Am J Clin Nutr* 63:646S–650S.

LSRO (Life Sciences Research Office). 1998. *Assessment of Nutrient Requirements for Infant Formulas*. Bethesda, MD: LSRO.

Owen CG, Whincup PH, Odoki K, Gilg JA, Cook DG. 2002. Infant feeding and blood cholesterol: A study in adolescents and a systematic review. *Pediatrics* 110:597–608.

Ryan AS, Wenjun Z, Acosta A. 2002. Breastfeeding continues to increase into the new millennium. *Pediatrics* 110:1103–1109.

SMDMC (Society for Medical Decision Making Committee). 1992. Proposal for clinical algorithm standards. Society for Medical Decision Making Committee on Standardization of Clinical Algorithms. *Med Decis Making* 12:149–154.

Vanderveen JE. 1991. The role of the Food and Drug Administration in regulating food products for children. *Ann N Y Acad Sci* 623:400–405.

2

Defining Safety for Infants

ABSTRACT

"Safety" refers to a reasonable certainty of no harm and is described by noting a range along a continuum, rather than as an absolute point or value (Food Additives Amendment, P.L. 85-929 of the Federal Food, Drug and Cosmetic [FD&C] Act, 21 U.S.C. §301). The relationship between biology and safety is mediated through the concepts of harm, benefit, and risk.

Manufacturers may propose the addition of a new ingredient to infant formulas by demonstrating the safety, not the efficacy (the capacity to produce an intended effect under the realistic situation of product use), of the proposed ingredient. Foods are generally considered to be inherently efficacious (with inherent sensory properties and nutrition) and, thus, efficacy is not a consideration in their safety assessment. In the case of infant formulas, this assumption is modified to some degree because it has been proposed that the products must be capable of sustaining physical growth for a specified period of time. Currently, however, manufacturers are not required to demonstrate the benefits of an individual ingredient in the product. Infancy is a uniquely vulnerable period that complicates the interpretation of safety guidelines. Not all organ systems are fully mature at birth, and as they undergo further development they are highly susceptible to nutritional inputs, illnesses, care practices, and other environmental inputs. Early infancy represents a period of growth and development when a successful outcome depends on the timely emergence of critical structures and developmental processes. The gastrointestinal, renal, and immune systems, as well as brain and neurological functions, could be affected by exposure to potentially harmful substances contained in infant formulas. Optimization of nutrition and minimization of exposure to potentially harmful substances in the food supply is of heightened importance during infancy. The committee concluded that there are six issues that must be considered as important safety issues when regulating infant formulas: (1) infant formulas are the sole or predominant source of nutrition for many infants, (2) formulas are fed during a sensitive period of

development and may therefore have short- and long-term consequences for infant health, (3) animals may not be the most appropriate model on which to base decisions of safety, (4) "one size fits all" food safety models may not work for all new additions to formulas, (5) infant formulas could be considered as more than just food, and (6) potential benefits, along with safety, should be considered when adding a new ingredient to formulas.

INTRODUCTION

A discussion on guidelines to ensure the safety of ingredients new to infant formulas requires a broad understanding of the concepts and models of safety regulations and an in-depth review of infancy as a unique period that requires unique safety measures. This chapter describes fundamental concepts of safety regulations, statistical considerations in assessing food safety, models of safety assessment (including the Novel Foods model of Health Canada), and special considerations for ensuring the safety of infants and regulating infant formulas.

U.S. REGULATORY AGENCIES

Several federal, state, and local agencies monitor the safety and quality of substances that are ingested and inhaled by the general public. Each agency operates within its own domain using its own set of rules and procedures, but the agencies work collaboratively on some issues to ensure the safety and quality of food, water, and air. The Food and Drug Administration (FDA) has the primary responsibility for regulating ingredients new to infant formulas and other agencies serve in peripheral roles.

FUNDAMENTAL CONCEPTS OF SAFETY REGULATIONS

The terms *safety, hazard, risk, harm,* and *benefit* are central to the full understanding of safety assessment. *Safety* refers to a reasonable certainty of no harm (21 U.S.C §348) (e.g., for food ingredients in the U.S. regulatory system) or, in some systems (e.g., for pharmaceutical drugs in the U.S. regulatory system), a reasonable balance between costs (harm) and benefits. Safety is an intellectual concept; it is *not* an inherent biological property of a substance. Safety is described by noting a range along a continuum, not an absolute point or value. The relationship between biology and safety is mediated through concepts of harm, benefit, and risk. As such, the concept of safety and its assessment is influenced by many different organizations, individuals, and intellectual disciplines.

A *hazard* generally refers to some substance or combination of substances (organic or inorganic, or in some cases psychological) that may, under some circumstances or for some individuals, produce undesired health-related outcomes. Nearly any substance can have the potential to produce an undesired health outcome to some individual under some circumstance. Exposure to a hazard, however, does not guarantee that an undesired outcome will occur.

The likelihood that such an outcome will occur given exposure to a hazard is what is referred to as the *risk* of that hazard. The risk of a hazard is not a general (main effect) property of the substance, but rather it is an interaction between the nature of the hazard, the circumstances of the exposure (e.g., the magnitude, the timing, and the specific features of the individual, such as health status, age, and susceptibility), and other such conditions that moderate the actual health impact of the hazard.

Harm refers to the nature of the undesired outcome (usually a health outcome) associ-

ated with a hazard and is often expressed in terms such as cost. Not all harm is the same, and not all individuals would assess the same outcome as having equivalent harm.

Opposite harms are the *benefits* of the addition of substances. The ratio of costs to benefits is a critical unit in some safety assessment systems. Cost-benefit ratios may apply to individuals or groups. For example, an individual may benefit from a treatment but may experience side effects. Another example is the case of iron supplementation to reduce infant risk of anemia. Some or all of the infants within a group may benefit from receiving additional iron, while some may be harmed (e.g., experience constipation); on average, however, the population that benefits from iron fortification will be larger than the population that experiences harm.

Members of the general public, special interest advocates, lawmakers, scientists, leaders of government agencies, and industry representatives play significant roles in establishing safety guidelines. The public makes certain demands for lowering food safety risks (whether real or perceived risks as a result of misinformation) and expresses its concerns either through consumer organizations or through individual contact with appropriate government agencies. Consumer organizations may voice such concerns in a focused manner. Lawmakers weigh these concerns and, where appropriate, engage in debates that may result in new laws and regulations. In the process of establishing formal policy, other individuals and organizations often enter into the debate to influence the final statements of safety regulation. For example, scientists or professional organizations may contribute important information that derives from scientific studies, economists may provide information about the costs of implementing certain safety standards, and industry representatives may describe the impact of the regulation on manufacturers. Once laws are enacted, regulatory agencies are entrusted with the responsibility of developing, implementing, and enforcing regulations.

STATISTICAL CONSIDERATIONS IN FOOD SAFETY DETERMINATION

Among adults, guidance concerning the clinical relevance of differences in some physiological parameter (e.g., blood pressure) is derived from a body of accumulated evidence that provides a rationale for a clear definition of pathological state (e.g., hypertension). In testing an ingredient new to an infant formula, it is unlikely that investigators would detect any clear evidence of disease (which should have been ruled out by preclinical testing). Instead, more subtle differences in physiology or development may appear that lack sufficient evidence to inform clinical judgment. Investigators must determine whether a difference (e.g., level of growth) has an immediate health consequence for the infant and the level of difference that matters for the long term (e.g., a growth deficit associated with a particular ingredient rapidly disappears when other foods are added to the diet). In the absence of sufficient evidence for clinical judgment, investigators may be forced to utilize statistical or analytical approaches as the basis for making judgments about safety.

The process of establishing the safety of food products, especially infant formulas, is complex and requires empirical evidence from many disciplines. Each step in the process requires the application of the highest standards, whether using methods of bioassay, nutritional analysis, or basic chemistry. Eventually studies involving human subjects (particularly in the case of infants) must be conducted in order to demonstrate the product's safety for the human consumer. Studies involving humans are almost always conducted as randomized clinical trials and standard methods of design and analysis are followed.

The most typical analytical approach to interpreting the data from scientific studies, including clinical trials, is the statistical significance test, also known as the null hypothesis significance test (NHST). This approach, which has recently come under much scrutiny and debate, formally

applies a set of principles for establishing a "rule of evidence" in scientific inquiry. In the simplest terms, NHST provides a set of rules for decision making under uncertainty.

First, a set of two mutually exclusive alternative conditions is specified. For example, the addition of substance X to an infant formula either: (a) hinders the ability of the infant to maintain proper physical growth, or (b) it does not hinder proper physical growth. Second, a set of risk probabilities are specified (the "alpha level" of the test and the "power" of the test), which allows the researcher to control the probabilities of drawing an incorrect inference. Third, a set of assumptions is specified that, taken together with the null and alternative hypotheses, allow the complete specification of the behavior of some statistical index.

From this model one can specify a set of decision rules to draw some conclusions based on the empirical results of the experiment. The result of such a statistical test procedure does not establish with certainty the "true state of nature," but rather it expresses a degree of confidence that one of the two states is not likely to occur. The randomized clinical trial and associated NHST are the mainstays of certain safety and efficacy approaches, such as the FDA drug trials described later, but they have certain potential limitations in their application to the safety of ingredients new to infant formulas.

The first limitation is that NHST lacks a certain degree of direct applicability. The basic concept underlying the safety of an ingredient added to infant formulas is the "reasonable certainty of no harm" concept without a requirement for the demonstration of benefit. NHST, however, is generally formulated to demonstrate the superiority of one condition versus another. The fundamental idea in the formulation of reasonable certainty of no harm is one of equivalence, not of difference. While one can manipulate the null and alternative hypothesis in this circumstance (e.g., to make the "no difference" condition the alternative hypothesis), the resulting formulation is awkward and shifts the probabilistic control of the important error rate to that of power rather than to the more direct alpha level of the resulting test. Due to its highly unusual nature, it is likely that this test's results will not be properly understood and interpreted.

The second limitation is that NHST is concerned with demonstrating a difference rather than with the size or importance of the difference. In a number of scientific disciplines, most notably psychology, there has been a shift in emphasis from statistical significance to clinical significance. When formulated as clinical significance, the question becomes whether the difference that is detected by NHST is one that has any practical health consequences from the perspective of the individual receiving the treatment. Thus it is recognized that while a very small (potentially clinically inconsequential) difference can be detected by NHST (particularly in very large samples), the most important question is to determine whether the difference has any health implications.

In order to address these different questions, a number of approaches to testing have been adopted that include the following features:

- a greater focus on effect size estimates,
- the use of confidence intervals as the primary way of reporting the results of experiments, and
- the use of alternative statistical tests that are not based on the sample mean as the primary way of characterizing populations.

One approach to determine clinical significance is the use of dominance statistics (Cliff, 1993). In this approach it is asked, in a probabilistic manner, how likely is it that an individual chosen at random from the group receiving treatment A will score better than an individual chosen at random from the group receiving treatment B. Using the dominance

statistic approach, the question of weight maintenance might be approached as determining the probability that an infant drawn at random from a group consuming the new formula would weigh less than an infant drawn at random from a group fed the existing formula. If this probability were not too extreme, it might argue for the safety of the new addition relative to that of a formula without that addition.

An alternative approach to NHST may also be considered. For example, Seaman and Serlin (1998) developed methodologies that allow the direct demonstration of equivalence (as opposed to the subtly different approach of NHST that is oriented to showing differences). Such approaches may be applied to establish rules to determine the safety of ingredients new to infant formulas.

In assessing the safety of ingredients new to infant formulas, investigators must consider whether the NHST approach is the most appropriate method to analyze empirical data or whether an approach that more directly assesses effect sizes, clinical significance, dominance, or equivalency is preferred. Of particular importance in the selection of a proper analytical technique is the question of whether any decrease in infant growth rate can be tolerated. If slower growth rates or other minor differences in physiology or function cannot be tolerated, then investigators may wish to demonstrate equivalency, rather than merely detect differences. In addition, the error rate that one is willing to tolerate must be carefully considered rather than routinely adopting the traditional 0.05 alpha level. It is the alpha level that indicates the "societal" values for the acceptance of risk and harm.

MODELS OF SAFETY ASSESSMENT

Virtually all other models used to assure the safety of ingested or inhaled substances are variations of the food and drug safety models (described below): the nutrients model based on dose-response relationships; the in-market monitoring and surveillance model; the novel foods and food additives models, based on a reasonable certainty of no harm; and the drug model, based on risk-benefits assessments.

Food Models

Nutrients Model

The Dietary Reference Intakes (DRIs) are a set of quantitative reference values for nutrient intake to be used for planning and assessing diets, and they are based on risk assessments. One of the DRIs, the Tolerable Upper Intake Level (UL), uses risk assessment approaches. The UL uses a substantially different approach from the one used for food additives in that it does not address any particular food product or ingredient, yet it provides useful information on the magnitude of intake of nutrients for individuals as a function of age, gender, pregnancy and lactation status, and other such factors. At the heart of the DRI methodology is a dose-response relationship (e.g., the examination of a targeted health outcome as a function of the intake of the nutrient in question). The UL is the level of intake at which one would expect virtually no risk of an adverse health outcome for almost all healthy individuals in the population. There would be an increased risk of an adverse health outcome if more than the UL were consumed. The actual risk assessment methodology of the DRI approach is quite complex; it is described in *Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients* (IOM, 1998) and in each of the nutrient-specific DRI reports, most recently in *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate* (IOM, 2004).

In-Market Monitoring and Surveillance

A different approach to ensuring food safety is in-market monitoring and surveillance. For instance, once an infant formula is released for general consumption, FDA requires that the manufacturer maintain and analyze records of consumer complaints. Further, the manufacturer is required to report to FDA cases where the evidence suggests a reasonable possibility of a link between consumption of the formula and an infant illness, death, or a reduction in intake of required nutrients. This is currently a passive system that merely requires that a caregiver report a suspected case, but it is one more mechanism by which the safety of infant formulas can be monitored. Details of in-market monitoring and surveillance are discussed in Chapter 7.

Novel Foods Model

In 1994 Health Canada issued the *Guidelines for the Safety Assessment of Novel Foods* (Health Canada, 1994) to assist in the design of premarket notifications for novel foods. The manufacturer must submit a request to Health Canada 45 days prior to the sale or advertising for sale of any novel food under specific requirements laid out in Division 28 of the Food and Drug Regulations (Canada, 2001). Where it is determined that data of a scientific nature are required to support the safety of the product, a safety assessment may include an evaluation of microbiological, molecular, chemical, nutritional, and toxicological endpoints in preclinical and clinical studies. Specifically, the assessment may evaluate the process used to develop the novel food (molecular, chemical, and microbiological), the comparison of the characteristics of the novel food with that of its traditional counterpart (nutritional and toxicological), safety issues related to a source with no history of use in the food supply (nutritional and toxicological), and the potential allergenicity from proteins introduced into the food (nutritional). Health Canada may request additional information to assess the safety of the novel food and, if satisfied, will notify the manufacturer that the information is sufficient and the product is safe for consumption.

Food Ingredients Model

The food safety evaluation of food ingredients and associated regulations in the United States is based on "reasonable certainty of no harm." Sections 201(s), 201(z), 409, and 412 of the FD&C Act and FDA's *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food*, also known as the Redbook[1] (OFAS, 2001, 2003), are the regulations and guidelines that are used to assess the safety of food ingredients and infant formulas.

Regulatory oversight of the addition of new ingredients to infant formulas is governed largely by two processes: the Food Additive Petition and the Generally Recognized as Safe (GRAS) Notification. It is important to note that these procedures are to ensure the safety (a reasonable certainty of no harm)—not the benefits—of the proposed ingredient. A *food additive* is any product added to a food that is not generally recognized as safe by qualified experts (see Box 4-1 in Chapter 4 for the complete definition). The food additive petition

[1]This FDA document should not be confused with the American Academy of Pediatrics' Red Book of childhood infectious diseases.

process requires that the manufacturer file a petition with FDA that provides all available data on the safety of the product and proposes the conditions under which such an additive may be safely used. FDA may issue a safety declaration upon review of the petition. In this case it is FDA that makes the affirmative declaration of the safety of the additive in the context of its proposed use.

By contrast, the GRAS Notification process requires that the manufacturer make the initial declaration of the safety of the product based on consensus by qualified experts. FDA then reviews the notification and, if all of its questions are satisfactorily answered, the agency issues a letter of no objection. Because this process has become the primary route of introduction of new ingredients to infant formulas, the GRAS Notification process is reviewed in greater detail in Chapter 4.

Infant formulas are the sole source of nutrition for many infants and, therefore, one step is required for the approval of modifications to formulas that is not required for other foods. Manufacturers seeking to market a new infant formula need to comply with regulations under Section 412 of the FD&C Act. New regulations under that section of the FD&C Act have been proposed that would require manufacturers to demonstrate that the formula containing the new ingredient in the matrix in which the product is delivered is capable of sustaining physical growth and development over 120 days, the period when the formula is likely to be the sole source of infant nutrition.

In 1982 FDA issued, and later updated, the Redbook (OFAS, 2001, 2003).[2] These guidance documents were prepared to assist in the design of protocols for animal studies conducted to test the safety of food ingredients and include detailed guidelines for testing the effects of food ingredients on mothers and their developing fetuses. However, due to the special conditions surrounding infancy described below, special considerations need to be taken into account when applying the Redbook in the case of infant formulas.

As mentioned previously, since virtually all models of safety determination are based on empirical evidence, only minor variations of the basic models seen in the FDA food safety determination system are possible. These differences are based more upon emphasis and implementation than on any profound differences in methodology. One major exception to this uniformity is the food safety model based upon the "reasonable certainty of no harm" concept. As opposed to other models where benefits from the addition of the new ingredient need to be demonstrated, in that model safety is seen as no harm and no proof of beneficial effects is needed. As discussed further in Chapter 4, the committee believes that for infant formulas, the concepts of efficacy (benefit) and safety are not always mutually exclusive because of the uniqueness of the infant population and, therefore, potential benefit should be considered when allowing new ingredients to be added to infant formulas.

Drug Safety Model

Structurally the FDA drug approval process closely resembles the food additive model of food safety. A manufacturer that wishes to market a new drug submits a petition to FDA,

[2]The original Redbook (Redbook I) was published in 1982, revised in 1993, and updated in 2001 (draft Redbook II). In 2000, FDA released a revised version of the publication as *Redbook 2000: Toxicological Principles for the Safety Assessment of Food Ingredients*. However, some chapters in Redbook 2000 have not yet been revised, so both the draft Redbook II and Redbook 2000 are used as guidance documents when conducting animal studies. Appendix C lists the contents of the draft Redbook II and Redbook 2000 and indicates which chapters in Redbook 2000 have been updated.

along with a specified set of empirical evidence concerning the product. FDA (with advice from an established panel of experts) evaluates the evidence and, if satisfied that the drug meets its regulatory criteria, approves the drug for commercial use. In the drug approval process, however, the evidentiary basis for the decision is quite different from that employed in the food additive model where evidence of benefit is not necessary.

First, the applicant must offer evidence through one or more clinical trials of the efficacy of the drug. That is, there must be clear and cogent evidence that the drug does what it claims to do. In addition, it must be shown to have the same effect as the current standard treatment of the condition being studied. Second, side effects (e.g., adverse reactions) of the drug must be carefully studied and reported. The criterion for approval of the applicant product is then based upon an assessment of the benefit:risk ratio.

Other Safety Models

As noted above, virtually all other models used to assure the safety of substances ingested or inhaled are variations of the food and drug safety models. Given the number of regulated substances (e.g., air, water, lead) and the number of agencies charged with regulating them at the federal, state, and local level, it is not surprising that numerous variations in safety determination practices exist. Most of these differences, however, are at the technical level rather than at the conceptual level (e.g., setting the standard for lead is conceptually very much like the establishment of upper boundaries in the DRI process).

SPECIAL CONSIDERATIONS FOR INFANT FORMULAS

Infancy as a Vulnerable Period

Dealing with infancy makes interpretation of safety guidelines particularly difficult. Infancy is a period of very rapid development, and change is the rule rather than the exception. Infants are nonverbal and cannot report their own experiences, and parents may not be able to accurately interpret the signals that infants provide. It is reasonable to expect that any harmful effects from an ingredient might be subtle. Throughout this report, the committee describes the period of infancy as so special and the consequences of any harm so great that the public should be unwilling to tolerate any harm due to the addition of an ingredient new to infant formulas, even if there are benefits to most who would be exposed to the new ingredient.

At birth, infants make a dramatic transition from a highly controlled prenatal environment where oxygen and nutrients are delivered directly from the mother to infant via the blood supply, to one that involves enteral feeding and thus requires an efficient gastrointestinal system and coordination of a wide range of functions. Not all organ systems are fully mature at birth, and as they undergo further development, they are highly susceptible to environmental inputs. Early infancy represents a period of growth and development when a successful outcome depends on the timely emergence of critical structures and developmental processes. At the same time, infancy is a period of heightened vulnerability to nutritional inputs, illnesses, care practices, and other environmental influences. Depending on the tissues involved and the timing and severity of insults, effects may be irreversible or the ability to compensate for differences may be limited. Furthermore, infants have a higher food consumption rate than adults when expressed on a per kilogram body-weight basis, and they typically rely on a sole nutrient source (human milk or milk substitutes) in the early months of life. Thus optimization of nutrition and minimization of exposure to potentially harmful

substances in the food supply is of heightened importance during infancy. Examples of the various systems that could be affected by exposure to potentially harmful substances contained in infant formulas are described below.

Gastrointestinal and Renal Function

With the exception of exocrine pancreatic function and bile acid metabolism, the gastrointestinal tract is anatomically and functionally fully developed in infants born at term, and dietary contents do not influence their development. Trophic factors can be found in human milk and in the gastrointestinal tract, but their function is unclear. As these factors are more fully identified and their structure, composition, and physiological effects understood, it is possible that one or several may have effects on infant organ systems.

Similar to the gastrointestinal tract, development of renal function is not influenced by dietary content. Glomular filtration does not approximate adult values until about 3 years of age, and tubular reabsorption and urine acidification reach normal values at several months of age. However both are sufficient for healthy term infants, but contribute to fluid and electrolyte abnormalities in infants who are ill and in those who are fed an inappropriate diet.

Immune Function

The infant immune system is not fully mature at birth; it has deficits in the ability to prevent invasion of pathogens and to respond to antigens. Of particular concern in the context of ingredients new to infant formulas is the increased permeability of the gut mucosal barrier in the presence of inflammation or infection or if the integrity of the epithelial cell layer is disrupted. The increased permeability allows macromolecules to be absorbed, which stimulates allergic responses to food proteins.

Brain and Behavior

There is rapid development of both brain and behavior during the first year of life. Both subcortical and cortical central nervous system (CNS) structures mature and allow the appearance of critical developmental functions, such as visually guided reaching, face recognition and orientation toward faces, explicit and working memory, focused attention, and inhibitory control (Johnson, 2001; Nelson, 1995). The quality of the young infant's nutritional intake can be an important influence on CNS development and function (Rao and Georgieff, 2000; Wauben and Wainwright, 1999). For example, iron deficiency anemia in the first year interferes with the development of functional CNS processes, such as alteration in the number of dopamine receptors or degree of myelination (Lozoff et al., 1998; Wauben and Wainwright, 1999). These functional changes in turn can have long-term cognitive and behavioral consequences that cannot be compensated for by subsequent nutritional interventions (Lozoff et al., 1998).

A similar process is also seen with regard to behavioral development. The infant's increasing behavioral competencies and patterns of social-emotional functioning during the first year can impact upon critical developmental mediators, such as patterns of parent-child relations, stress reactivity, initiation of self-regulation skills, and extent of infant interactions with the larger environment (Gunnar, 2000; Ruff and Rothbart, 1996). Interference with the development of these critical mediators also can have long-term consequences for later functional competence (Wachs, 2000). Since a large proportion of the infant's nutrition

during the first year may be supplied by formulas, changes to formulas that impact upon normally occurring brain and behavioral developmental patterns can have potential long-term consequences.

Important Safety Considerations

Most of the principles that govern the safety of ingredients new to infant formulas derive from the same principles that govern food safety for older children and adults. The committee concluded that the six issues described below and summarized in Box 2-1 must be considered as important safety issues when regulating infant formulas.

1. *Infant formulas are the sole or predominant source of nutrition for many infants.* Even for infants for whom formulas are a supplement to human milk, formulas can constitute a major source of nutrition. For infants, formulas represent a much larger percentage of the total nutritional intake than is any single substance for the older child, adolescent, or adult. Given the extremely high dependence on this one source of dietary input, safety rules based upon models deemed appropriate for persons with a wide range of dietary inputs may be inappropriate.

2. *Formulas are fed during a sensitive period of development and may therefore have short- and long-term consequences for infant health.* The first 12 to 18 months of life is a period of extremely rapid growth and development for the human infant that is only beginning to be understood. The brain and neural system change dramatically during this period, as do other organ, cognitive, and social-emotional systems. These changes are thought to have long-range implications for the human—not all of which are expressed directly during infancy. The current safety models for infant formulas only look at relatively short-term outcomes and are narrowly limited to the maintenance of physical growth.

3. *Animals may not be the most appropriate model on which to base decisions of safety.* In the case of infants, limitations of using experimental animals as models are due not only to species differences, as in the case of adults, but also to developmental differences in animals versus infants. The differences in growth rates and the resulting differences in biological effects of ingredients could therefore accentuate the concerns that already exist when using animal models. Given these differences, animal models may not uncover possible threats to the long-term well-being of infants due to the addition of new ingredients; this issue is of critical significance if the models used are developmentally inappropriate.

4. *"One size fits all" food safety models may not work for all new additions to formu-*

BOX 2-1 Important Safety Considerations When Regulating Infant Formulas

1. Infant formulas are the sole or predominant source of nutrition for many infants.
2. Formulas are fed during a sensitive period of development and may therefore have short- and long-term consequences for infant health.
3. Animals may not be the most appropriate model on which to base decisions of safety.
4. "One size fits all" food safety models may not work for all new additions to formulas.
5. Infant formulas could be considered as more than just food.
6. Potential benefits, along with safety, should be considered when adding a new ingredient to formulas.

las. The basic models of food safety are based upon the assumption that nearly all individuals function basically in similar ways with regard to the safety of substances in their diet (although there are gross subtypes with different dietary needs, such as premature infants and infants with food allergies). However it may be speculated that there are more subtle underlying subgroups (e.g., genetic subtypes) for which new substances may or may not have the same safety characteristics as they would for individuals not in those subgroups. In clinical trials the minimum sample size required to detect systematic group differences in response to a new ingredient is likely to be entirely inadequate for subgroup analyses.

5. *Infant formulas could be considered as more than just food*. The rules governing the safety of infant formulas are regulated under the logic of infant formulas being a "food product," and there is no doubt that the primary purpose of infant formulas is nutrition. However infant formulas can also be seen as a potential delivery system for non-nutritional agents. This potential as a delivery system should cause one to consider the question of what are the appropriate (both legal and ethical) boundaries for additions to infant formulas. Consideration must be given to constructs that will appropriately set the limits and provide the definitions for an addition being "nutritional." The presence of the substance in human milk may not be a sufficient definition of a nutritional substance, and other factors, such as whether the substance is produced from genetically modified sources, need to be considered. In addition, the regulatory steps to follow to ensure the safety of a substance whose purpose is non-nutritional (e.g., a substance whose purpose is to reduce infant discomfort) need to be determined.

6. *Potential benefits, along with safety, should be considered when adding a new ingredient to formulas*. Food products are generally considered to be inherently beneficial or efficacious (with inherent sensory properties and nutrition) and thus efficacy (i.e., health benefits) has not been a consideration in the safety assessment of foods. In the case of infant formulas, this starting assumption may be modified through the additional requirement in proposed regulations (FDA, 1996) that the product be capable of sustaining physical growth for a specified period of time, that is, it is already required that the final formulated product be demonstrated to be efficacious from the point of view of physical development. (This proposed requirement, however, is usually stated as a safety criterion rather than as an efficacy criterion, that is, a lack of efficacy would be a risk to the organism and therefore a safety concern.)

Other than this proposed requirement for sustaining physical growth, however, the manufacturer would not need to demonstrate the benefits of any proposed new ingredient to infant formulas. Given the special circumstances of the use of infant formulas and the fact that is it virtually impossible to understand the long-term outcomes of adding any given ingredient (the clinical trials are, of necessity, of relatively short duration), prudence would seem to dictate that an ingredient not be added to an infant formula unless it can be shown that some benefit is accrued to the infant through its addition. Since the committee was not charged with addressing efficacy, it recommends that consideration be given to convening a group of experts to explore if benefit could be an appropriate requirement for adding a new ingredient to infant formulas.

SUMMARY

Infancy is a uniquely vulnerable period that complicates the interpretation of safety guidelines. Manufacturers may propose the addition of a new ingredient to infant formulas by demonstrating the safety of the proposed ingredient. They are not required to demonstrate the benefit of an individual ingredient in the product. Although the committee believes

that, in the case of infant formulas, efficacy is an important consideration, the committee did not discuss this issue because it was beyond its charge.

The six safety considerations discussed in this chapter are important when developing safety guidelines for adding ingredients new to infant formulas. In making recommendations in the chapters that follow, the committee considers these six special issues that set infant nutrition apart from that of toddlers, children, and adults.

REFERENCES

Canada. 2001. *Departmental Consolidation of the Food and Drugs Act and the Food and Drug Regulations*. Ottawa: Minister of Public Works and Government Services Canada.

Cliff N. 1993. Dominance statistics: Ordinal analyses to answer ordinal questions. *Psychol Bull* 114:494–509.

FDA (Food and Drug Administration). 1996. Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula. Proposed rule. *Fed Regist* 61:36153–36219.

Gunnar MR. 2000. Early adversity and the development of stress reactivity and regulation. In: Nelson CA, ed. *The Effects of Early Adversity on Neurobehavioral Development. The Minnesota Symposia on Child Psychology*. Vol. 31. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 163–200.

Health Canada. 1994. *Guidelines for the Safety Assessment of Novel Foods*. Ottawa: Food Directorate, Health Protection Branch, Health Canada.

IOM (Institute of Medicine). 1998. *Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients*. Washington, DC: National Academy Press.

IOM. 2004. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. Washington, DC: The National Academies Press.

Johnson MH. 2001. Functional brain development in humans. *Nat Rev Neurosci* 2:475–483.

Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. 1998. Behavior of infants with iron-deficiency anemia. *Child Dev* 69:24–36.

Nelson CA. 1995. The ontogeny of human memory: A cognitive neuroscience perspective. *Dev Psychol* 31:723–738.

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS. 2003. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

Rao R, Georgieff MK. 2000. Early nutrition and brain development. In: Nelson C, ed. *The Minnesota Symposia on Child Psychology. The Effects of Adversity on Neurobehavioral Development*. Vol. 31. Mahway, NJ: Lawrence Erlbaum Associates. Pp. 1–30.

Ruff HA, Rothbart MK. 1996. *Attention in Early Development. Themes and Variations*. New York: Oxford University Press.

Seaman MA, Serlin RC. 1998. Equivalence confidence intervals for two-group comparisons of means. *Psychol Methods* 3:403–411.

Wachs TD. 2000. *Necessary but Not Sufficient. The Respective Roles of Single and Multiple Influences on Individual Development*. Washington, DC: American Psychological Association.

Wauben IP, Wainwright PE. 1999. The influence of neonatal nutrition on behavioral development: A critical appraisal. *Nutr Rev* 57:35–44.

3

Comparing Infant Formulas with Human Milk

ABSTRACT

The vast majority of infants in the United States are fed human-milk substitutes by 6 months of age. This food source, although inferior to human milk in multiple respects, promotes more efficient growth, development, and nutrient balance than commercially available cow milk.

Manufacturers often add new ingredients to infant formulas in an attempt to mimic the composition or performance of human milk. However the addition of these ingredients is not without risks as a result of a range of complex issues, such as bioavailability, the potential for toxicity, and the practice of feeding formula *and* human milk within the same feeding or on the same day.

Assessing the safety of ingredients new to infant formulas by comparing the proposed formulas with human milk also presents both regulatory and research issues. From a research standpoint, clinical studies that assess the effects of new ingredients are difficult to design because infants cannot be randomized to consume formulas or human milk. Furthermore, there may be significant non-nutritional confounding variables between the groups, including factors related to which mothers choose to breastfeed. Finally, human-milk composition varies considerably among and within individuals over time, while the content of infant formulas generally remains constant.

From a regulatory standpoint, the effect of an ingredient new to infant formulas is usually driven by the manufacturer's desire to produce a product that mimics the advantages of breastfeeding. This motivation implies that formulas in their current state are less efficacious (e.g., neurologically or immunologically), although not necessarily unsafe, when compared with human milk. Thus the safety of any addition of an ingredient new to infant formulas will need to be judged against two controls: the previous iteration of the formulas without the added ingredient and human milk.

BACKGROUND

Multiple health organizations, including the World Health Organization (WHO, 2002), the American Academy of Pediatrics (AAP, 1997), the American Academy of Family Physicians (AAFP, 2003), the American Dietetic Association (ADA, 2001), the Institute of Medicine (IOM, 1991), the Life Sciences Research Organization (LSRO, 1998), the U.S. Department of Health and Human Services (HHS/OWH, 2000), Health Canada, and the Canadian Pediatric Society (Canadian Paediatric Society, 1998) endorse breastfeeding as the optimal form of nutrition for infants for the first year of life. Nevertheless the vast majority of infants in the United States are fed human milk substitutes by 6 months of age (Ryan et al., 2002). This food source, although inferior to human milk in multiple respects, promotes more efficient growth, development, and nutrient balance than commercially available cow milk. The American Academy of Pediatrics recommends that infants who are not breastfed should consume iron-fortified infant formulas rather than cow or goat milk until 12 months of age (AAP, 1997).

HISTORY OF THE DEVELOPMENT OF INFANT FORMULAS

Milk-Based Formulas

Human-milk substitutes existed before the modern age of formulas. Because some infants could not be fed by their mothers, humans adopted two methods for substitute feedings. The most obvious was the utilization of a surrogate mother (e.g., wet nurse), who would feed the child human milk. The alternative was to feed the child milk obtained from another mammal. The most frequently used sources were the cow, sheep, and goat (Fomon, 1993). Until the end of the nineteenth century, the use of a wet nurse was by far the safest way to feed infants who could not be breastfed by their mothers. As general sanitation measures improved during the latter part of the nineteenth century, and as differences in composition between human milk and that of other mammals were defined, feeding animal milk became more successful. However few infants survived until infant formulas based on cow milk with added water and carbohydrate were introduced. Box 3-1 lists the main landmarks in the

BOX 3-1 History of Commercially Available Infant Formulas in the United States

Cow-milk-based formulas
1867 – Formula contained wheat flour, cow milk, malt flour, and potassium bicarbonate
1915 – Formula contained cow milk, lactose, oleo oils, and vegetable oils; powdered form
1935 – Protein content of formula considered
1959 – Iron fortification introduced
1960 – Renal solute load considered; formula as a concentrated liquid
1962 – Whey:casein ratio similar to human milk
1984 – Taurine fortification introduced
Late 1990s – Nucleotide fortification introduced
Early 2000s – Long-chain polyunsaturated fatty-acid fortification introduced

Noncow-milk-based formulas
1929 – Introduction of commercially available soy formula (soy flour)
Mid 1960s – Isolated soy protein introduced

history of the development of infant formulas. Liebig's food for infants was marketed in 1867 and consisted of wheat flour, cow milk, malt flour, and potassium bicarbonate (Fomon, 2001). In 1915 a formula called "synthetic milk adapted" was developed with nonfat cow milk, lactose, oleo oils, and vegetable oils. This was the basis for modern commercially prepared formulas (Fomon, 1993).

The limitations of using nonhuman-mammalian milks as substitutes became clear. As early as 1545, people were concerned with the feeding of animal milks to babies. The *Boke of Chyldren* stated that "If children be fed the milk of sheep, then their hair will be soft as that of a lamb, but if they be fed the milk of the goat, the hair will be coarse" (Phaire, 1955, P. 18). There are, of course, far greater concerns about feeding animal milk to infants, such as folate deficiency (goat milk) and early onset hypocalcemic seizures and azotemia (cow milk).

By the early twentieth century it was clear that cow milk was most likely the best animal-milk base to work from, but that certain modifications were needed to make it safe and palatable for human infants. These modifications included:

- removing animal fat and substituting vegetable oils,
- diluting the protein content for the newborn's relatively immature renal tubular system, and
- adding or balancing minerals and vitamins (e.g., adding iron, adjusting the calcium: phosphorus ratio).

The process of modifying cow milk for large-scale production in the 1920s represented the birth of the infant formula industry. Since then new ingredients have been added for a variety of reasons. For example, iron was added in 1959 to reduce the risk of iron deficiency in formula-fed infants (Fomon, 1993), and long-chain polyunsaturated fatty acids (LC-PUFAs) were recently added in an effort to improve infant visual and cognitive development.

The protein content of formulas was a consideration from about 1935 onward. Early estimates of human-milk protein levels were higher than is now known, and it was believed that cow-milk protein was far inferior to human-milk protein. Formulas thus included high levels of protein (3.3–4.0 g/100 kcal). In the 1960s renal solute load began to be considered in the design of infant formulas, although infant formula regulations permit higher loads than are currently recommended by expert panels (no greater than 30 mosm/100 kcal) (Fomon, 2001).

Based on the recognition that human milk contains a predominance of whey proteins, while in cow milk, caseins are higher, formulas with a whey:casein ratio similar to human milk were introduced in 1962. By 2000 whey-predominant formulas were the most widely used milk-based formulas. These changes were made primarily based on composition rather than on functional measures (Fomon, 2001).

In 1984 taurine was added to infant formulas, based on at least a decade of studies that included composition, provisional essentiality, safety, and function in mammals (MacLean and Benson, 1989). Nucleotides were added to formulas with both compositional and efficacy claims in the late 1990s. They may act as growth factors and may have immunomodulating effects on immune defenses (Carver et al., 1991).

When considering new ingredients, manufacturers analyze every step in the production process, including raw materials (availability, source, and purity), processing methods, packaging, storage conditions and shelf life, methods of home preparation, and potential for misuse. Chapter 4 provides a discussion of these manufacturing considerations and their relevance to the regulatory process.

These considerations continue today as manufacturers attempt to alter infant formulas to imitate human milk in either composition or performance and to address the nutritional needs of specific infant populations (e.g., those with cow-milk allergy, metabolic abnormalities, and prematurity) (Benson and Masor, 1994). This chapter is concerned with infant formulas that are being altered to mimic composition or performance of human milk; it does not address the nutritional needs of specific infant populations.

Nonmilk-Based Formulas

Soy-based formulas were developed for infants perceived to be intolerant of cow-milk protein. The first soy formulas were commercially available in 1929 (Abt, 1965). These formulas were made with soy flour and were not well accepted by parents, who complained of loose, malodorous stools, diaper rash, and stained clothing. In the mid-1960s isolated soy protein was introduced into formulas. These formulas were much more like milk-based formulas in appearance and acceptance. However the preparation of isolated soy protein resulted in the elimination of most of the vitamin K in the soy, and a few cases of vitamin K deficiency were reported. The occurrence of nutrient deficiencies in infants fed milk-free formulas contributed to the development of federal regulations concerning the nutrient content of formulas (Fomon, 1993). Soy formulas now account for about 40 percent of formula sales in the United States. Some parents want to avoid cow-milk protein in the diet and thus wean directly to soy without any reported intolerance to cow-milk formulas. While formulas containing extensively hydrolyzed protein have long been available for infants with allergy to intact cow-milk protein, formulas with protein that is not as completely hydrolyzed have recently been introduced for normal-term infants.

CHALLENGES OF MATCHING HUMAN-MILK COMPOSITION AND BREASTFEEDING PERFORMANCE

Infant formula manufacturers have made changes to formulas in order to match either human milk composition or breastfeeding performance (Benson and Masor, 1994). The term "breastfeeding performance" is used because, with the exception of one study of preterm infants (Lucas et al., 1994), all other studies comparing human milk with formulas involved breastfeeding—not providing human milk from a bottle.

Matching Human-Milk Composition

Historically one approach to match human-milk composition is to add new ingredients (see Appendix B for the composition of formulas and human milk). This turns out to be a quixotic quest since human milk is a complex body fluid that is variable not only among individuals, but within an individual over time. In addition, it contains components, such as live cells and bioactive compounds, that either cannot be added to formulas or cannot survive a shelf life. Finally, not all human-milk constituents are essential; some, like LC-PUFAs, docosahexaenoic acid (DHA), and arachidonic acid (ARA), can be synthesized by term and preterm infants born at 33 weeks gestation (Uauy et al., 2000).

Manufacturers who wish to add some, but not all, ingredients found in human milk may defeat the purpose of the added nutrients or may potentiate negative interactions. Examples include the deleterious effect on growth when eicosapentaenoic acid is added without adequate DHA (Carlson et al., 1996) and the potential negative effect of adding polyunsatu-

rated fats and large amounts of iron without adding adequate antioxidants (Halliwell and Chirico, 1993; McCord, 1996).

The issue of the context or matrix in which nutrients are provided in milk remains a challenge to infant formula manufacturers as they try to match human-milk composition and breastfeeding performance (Benson and Masor, 1994). The matrix can highly influence the bioavailability of nutrients. In the simplest example, nutrients that are present in both milks may be present in different ratios. For many nutrients that do not interact chemically or compete for enzymatic or receptor binding sites, the relative amounts may not be important. However in situations where there is competition for enzymes (e.g., among *n*-3 and *n*-6 PUFAs) (Brenner, 1974) or receptor binding sites in the intestine (e.g., for zinc, iron, and copper), the relative proportions may have biological significance.

Manufacturers must also consider the form of the molecule in which a nutrient is presented to the intestine and its bioavailability. For example, the high bioavailability of iron from lactoferrin in human milk allows for a much lower concentration of iron in human milk (0.2–0.4 mg/L) compared with infant formulas (4.0–12 mg/L) and thereby decreases competition between iron and other divalent cations, such as copper and zinc (Lonnerdal and Hernell, 1994).

In the case of LC-PUFAs, care must be taken to ensure no toxicity from these compounds. Manufacturers must study the effects of fats, minerals, enzymes, or other factors on LC–PUFA bioavailability and processing. For example, newborn fat absorption can be highly variable because of the immaturity of several lipases, including pancreatic lipase (for review, see Hamosh, 1988). Human milk contains lipases that compensate for the lack of pancreatic lipases. Thus human-milk fat is more bioavailable than the vegetable oils found in infant formulas.

Finally, manufacturers must examine the effects of infant formulas in the context of mixed feedings (Ryan et al., 2002). Throughout the course of the day, an infant in the United States may consume both human milk and infant formulas in any number of combinations. For example, some infants of working mothers are breastfed during the morning and evening and fed formula during the day by a caregiver. Here the nutrients and their respective matrixes are kept quite separate and less interaction may be expected than in the situation where an infant is supplemented with formula directly after each nursing. In the latter case there is a theoretical concern that certain nutrients found in high concentration in infant formulas (e.g., iron) may interfere with the intended matrix delivery system found in human milk (e.g., lactoferrin). The nutritional consequence of mixed-feeding paradigms has not been adequately investigated, but should be targeted in future studies of the performance of infant formulas.

Matching Breastfeeding Performance

The alternative to matching human-milk composition is to match breastfeeding performance (Benson and Masor, 1994). Initially the goal of infant formulas was to match the growth rate of the breastfed infant. However over time it was recognized that breastfeeding may confer several other potential advantages to the infant (for review, see AAP, 1997), including:

- prevention of infectious diseases (Beaudry et al., 1995; Dewey et al., 1995),
- neurodevelopment (Mortensen et al., 2002), and
- protection from chronic diseases in childhood (Saarinen and Kajosaari, 1995; Shu et al., 1995).

These perceived and potential advantages of breastfeeding are the impetus behind many of the proposed addition of ingredients to infant formulas. Not all of these advantages are necessarily attributable to the nutritional content of human milk. Advantages resulting from a fundamentally different interaction between the nursing mother and her infant or to a selection bias of mothers who choose to breastfeed cannot be matched by simply adding nutrients to cow milk. It has been difficult to sort out which of the performance factors of breastfeeding are due to nutritional components and which are accounted for by social and psychological factors. Obviously, randomized trials assigning infants to breastfeed or formula feed are not ethically feasible.

Breastfeeding also confers certain risks to the developing infant, including potential nutrient deficiencies (Kreiter et al., 2000; Pisacane et al., 1995) and exposure to toxins secreted by the mother into her milk. Advantages and risks are discussed in detail below.

PERFORMANCE ADVANTAGES OF BREASTFEEDING

Breastfed infants have different growth characteristics compared with formula-fed infants. They grow at slightly different rates and have a different body composition (Butte et al., 1990; Heinig et al., 1993) and may have a lower risk for later obesity (Gillman et al., 2001; Singhal et al., 2002). (These characteristics are discussed in greater detail in Chapter 6.) Given the great interest in the effect of early nutrition on metabolic setpoints that may affect the child's risk for adult diseases (e.g., the early origins of chronic disease hypothesis) (Barker et al., 2002) and the increasing incidence of early insulin resistance, obesity, and type II diabetes in teenagers, future research should concentrate on whether breastfeeding is protective.

As discussed earlier, breastfed infants absorb fat better than formula-fed infants due to the presence of lipases in human milk that are not present in cow milk (Hamosh, 1988). The healthy breastfed infant consumes less milk (approximately 85 kcal/kg body weight/day) during the first months of life than the same infant given ad libitum infant formula (100 kcal/kg/day; Heinig et al., 1993). The breastfed infant continues to consume approximately 10 fewer kcal/kg/body weight calories than the formula-fed infant. The breastfed infant has a lower total energy expenditure (Butte et al., 1990) and a slower growth rate (Butte et al., 1990; Heinig et al., 1993). In addition, there is less gastro-esophageal reflux in breastfed infants, most likely due to a more rapid gastric emptying time, resulting in less loss of intake. Some of the trophic and metabolic factors that promote the characteristic nutrient handling and growth of the breastfed infant are listed in Table 3-1.

Breastfed infants, compared with formula-fed infants, have a lower incidence of infectious diseases, such as diarrhea (Popkin et al., 1990), otitis media (Duncan et al., 1993), and lower respiratory tract illness (Wright et al., 1989). The effect is particularly profound in the developing world, but studies show clear advantages in the developed world as well (Wright et al., 1989). The effect extends beyond breastfeeding itself to when human milk is administered without the infant nursing from the mother. For example, preterm infants fed human milk by nasogastric tube in the newborn intensive care unit have a lower rate of necrotizing enterocolitis (Lucas and Cole, 1990). Moreover, the presence of the close contact between the mother and child stimulates the mother to make antibodies against bacteria colonized in the infant and to secrete these antibodies in her milk.

Human milk has multiple components that likely mediate this anti-infectious, immunologically enhancing effect. These include secretory immunoglobulin A, lactoferrin, lysozymes, intact cellular components, and oligosaccharides. A comprehensive list of compounds found in human milk by class of ingredient is shown in Table 3-2.

TABLE 3-1 Unique Factors in Human Milk That Positively Affect Nutritional Status and Somatic Growth

Ingredient	Class of Ingredient	Function	Reference
Amylase	Enzyme	Polysaccharide digestion	Howell et al., 1986
Epidermal growth factor	Growth factor/hormone	Gastrointestinal growth/ differentiation	Donovan and Odle, 1994; Dvorak et al., 2003; Howell et al., 1986
Erythropoietin	Growth factor/hormone	Red cell production; possible growth factor for gut and central nervous system	Kling, 2002
Insulin	Growth factor/hormone	Anabolic hormone promotes carbohydrate, protein, and fat accretion	Donovan and Odle, 1994
Insulin-like growth factor-I	Growth factor/hormone	Primary growth hormone of late fetal/neonatal period	Donovan and Odle, 1994
Insulin-like growth factor-II	Growth factor/hormone	Unknown function; thought to be weak growth hormone	Donovan and Odle, 1994
Lactoferrin	Carrier protein	Increases efficiency of iron delivery	Howell et al., 1986
Lipase	Enzyme	Triglyceride hydrolysis	Howell et al., 1986
Nerve growth factor	Growth factor/hormone	Neuronal growth/ differentiation	Donovan and Odle, 1994
Proteases	Enzyme	Unknown if active in protein hydrolysis	Howell et al., 1986
Relaxin	Growth factor/hormone	Regulates morphological development of the nipple	Donovan and Odle, 1994
Transforming growth factor-alpha	Growth factor/hormone	Gastrointestinal growth	Donovan and Odle, 1994; Dvorak et al., 2003

TABLE 3-2 Unique Factors in Human Milk with Anti-Infective or Immunological Properties

Ingredient	Class of Ingredient	Function	Reference
Antiproteases (e.g., secretary immunoglobulin A and trypsin inhibitor)	Enzyme	Inhibits breakdown of anti-infective immunoglobulins and enzymes	Howell et al., 1986; IOM, 1991
Arylsulfatase	Enzyme	Degrades leukotrienes	Hanson et al., 1988
Catalase	Enzyme	Degrades hydrogen peroxide; protects against bacterial breeches of intestinal barrier	Lindmark-Mansson and Akesson, 2000
Fibronectin	Opsonin	May present debris to macrophages	IOM, 1991; Mestecky et al., 1990
Free fatty acids	Lipids	Antiviral (coronavirus); antiparasitic (*Giardia, Entamoeba*)	Mestecky et al., 1990
Granulocyte-colony stimulating factor	Cytokine	Causes endothelial cell migration and proliferation	Wallace et al., 1997
Hemagglutinin inhibitor	Opsonin	Prevents bacterial adherence	Neeser et al., 1988

Continued

TABLE 3-2 continued

Ingredient	Class of Ingredient	Function	Reference
Histaminase	Enzyme	Degrades histamine	Hanson et al.,1988
Immunoglobulin G	Immunoglobin	Immune protection	Howell et al., 1986; IOM, 1991
Interleukin-1-beta	Cytokine	Activates T-cells	Mestecky et al., 1990
Interleukin-6	Cytokine	Enhances immunoglobulin A and C-reactive protein production	Mestecky et al., 1990
Interleukin-8	Cytokine	Chemotaxis	Maheshwari et al., 2002
Interleukin-10	Cytokine	Decreases inflammatory cytokine synthesis	Goldman et al., 1996
Lactadherin	Protein	Prevents rotavirus binding	Peterson et al., 2001
Lactoferrin	Carrier	Anti-infective; may prevent iron from being bioavailable to microbes	Howell et al., 1986; IOM, 1991
Leukocytes	Live cell	Cytokine production by T-cells; direct in vivo roles of B-cells, macrophages, and neutrophils	IOM, 1991; Mestecky et al., 1990
Lipases	Enzyme	Releases bacteriostatic and bacteriocidal free fatty acids	Howell et al., 1986; IOM, 1991
Lysozyme	Enzyme	Bactericidal	Howell et al., 1986; IOM, 1991
Macrophage colony stimulating factor	Cytokine	Macrophage proliferation	Goldman et al., 1986
Mucin	Protein	Inhibits *E. coli* binding to gut epithelium	Peterson et al., 2001
Oligosaccharides, polysaccharides, gangliosides	Carbohydrates, glycoconjugates	Receptor analogs block binding of enteric bacteria; growth promoters for *Lactobacillus*	Coppa et al., 1999; IOM, 1991; Rivero-Urgell and Santamaria-Orleans, 2001
Peroxidases	Enzyme	Bactericidal	Howell et al., 1986; IOM, 1991
Platelet activating acetyl hydrolase factor	Enzyme	Catabolizes platelet activator factor	Furukawa et al., 1993
Prostaglandin E2, F2-alpha	Prostaglandin	Intestinal cytoprotection	Howell et al., 1986
Ribonuclease	Enzyme	Prevents viral replication	Nevinsky and Buneva, 2002
Secretory immunoglobulin A	Immunoglobulin	Immune protection (broad spectrum antiviral, antibacterial, antiparasitic)	Howell et al., 1986; IOM, 1991
Soluble intracellular adhesion molecule-1	Cytokine	Alters adhesion of viral or other molecules to intestinal epithelium	Xyni et al., 2000
Transforming growth factor-beta	Cytokine	Produces immunoglobulin A and activates B-cells	Bottcher et al., 2000
Tumor necrosis factor-alpha	Cytokine	Mobilizes amino acids	Mestecky et al., 1990
Uric acid	Small molecular-weight nitrogenous compound	Antioxidant	Van Zoeren-Grobben et al., 1994

The neurodevelopmental advantages of breastfeeding or supplying infants with human milk have received significant amounts of attention (Lucas et al., 1998; Morrow-Tlucak et al., 1988; Mortensen et al., 2002; Wang and Wu, 1996). Indeed, the primary impetus for adding LC–PUFAs to infant formulas is their postulated effect on brain development. The general research on breastfeeding, human milk, and neurodevelopment is fraught with confounding variables that have prevented pinpointing specific nutrients that are responsible for the putative effect. Overall it appears that breastfed infants have modest improvements in cognitive, motor, and visual status up to the age of 8 years, but it is unclear whether any early effects disappear over time (for review, see Grantham-McGregor et al., 1999). The degree of neurodevelopmental advantage is directly related to duration of breastfeeding (Mortensen et al., 2002). However critics of the literature point out that there may be fundamental differences not only between mothers who do or do not choose to breastfeed, but also between those who choose to breastfeed for a longer rather than shorter time period. These selection biases may be based on characteristics (e.g., maternal IQ, education, and socioeconomic status) that may confer independent positive effects on the neurodevelopment of the infant. Furthermore, patterns of parent-child interactions may be different in those who breastfeed longer; these interactions may have effects on development.

Just as it is difficult to separate out the confounding social factors among those who do and do not choose to breastfeed, it is also difficult to isolate the role of nutrition alone in the assessment of the positive effects. This is because very few individuals bottle-feed their infants human milk and, when this is done, it is frequently for medically extenuating circumstances (e.g., prematurity). Thus one cannot expect to rely on randomized trials of breastfeeding versus formula feeding or breastfeeding versus bottle feeding of human milk to sort out the nutritional effects of human milk on the developing brain. The only trial that approached this issue was conducted by Lucas and coworkers (1994), where preterm infants received either human milk or term infant formula by gavage tube during their early weeks. Infants fed bottled human milk had higher mental and psychomotor development indices 18 months after hospital discharge. However it should be reiterated that these were premature infants and that they were not randomized to their particular diets.

Nevertheless there are reasons to think that the provision of human milk, based on its composition, is good for the human brain. Human milk contains LC–PUFAs (e.g., DHA and ARA) that are important for synaptogenesis in the visual system. However studies assessing the addition of these ingredients to cow-milk formula have not resulted in consistent effects. Some demonstrated enhanced visual acuity and speed of processing in infants fed the supplemented formulas (Uauy et al., 1990; for review, see Uauy-Dagach and Mena, 1995). The positive effects on visual acuity have been found most often in premature infants, who are arguably more deficient of these fats. There may be effects on cognitive outcome, although the effects are inconsistent, particularly in term infants (Auestad et al., 2001; Wroble et al., 2002). The reason for these inconsistent effects might be that these compounds do not work alone; rather the matrix of human milk includes general growth factors and specific neural growth factors (see Table 3-3). If there is a positive effect on neurodevelopment, it is likely that these factors work in concert with each other.

Finally, there is epidemiological evidence that breastfeeding protects infants from certain childhood diseases at older ages, including atopy/allergy (Kull et al., 2002; Saarinen and Kajosaari, 1995), obesity (Gillman et al., 2001; Singhal et al., 2002), and childhood leukemia/lymphoma (Shu et al., 1995). The biological mechanisms of the positive effects are not always clear, but may relate to avoidance of exposure to antigenic proteins found in cow milk, particularly in relation to allergy. The lack of clear biological mechanisms makes it more difficult to resolve conflicting results, such as those recently indicating an increased

TABLE 3-3 Unique Factors in Human Milk That May Positively Affect Neurodevelopment

Ingredient	Class of Ingredient	Function	Reference
Choline	Amino acid	Neurotransmitter synthesis	Zeisel et al., 1986
Insulin-like growth factor-1	Growth factor/hormone	Neuronal growth/ differentiation	Cheng et al., 2003; Donovan and Odle, 1994
Long-chain polyunsaturated fatty acids	Essential/semiessential fat	Visual acuity	Uauy-Dagach and Mena, 1995
Nerve growth factor	Growth factor/hormone	Neuronal growth/ differentiation	Donovan and Odle, 1994
Oligosaccharides (fucose, mannose, *n*-acetylglucosamine, sialic acid)	Carbohydrates	Neuronal cell-cell communication	Hynes et al., 1989

risk of atopy (Sears et al., 2002) and eczema (Bergmann et al., 2002) in large cohorts of breastfed infants.

RISKS OF BREASTFEEDING

Breastfeeding is not without potential nutritional risks. The best documented risks include iron deficiency (Duncan et al., 1985; Pisacane et al., 1995), vitamin D deficiency (Kreiter et al., 2000), and exposure to environmental toxins. The inability to sustain growth due to the low energy density of milk is relatively rare in the first 4 months of life in the breastfed infant. However there is great variability in the protein-energy density of human milk. Energy values may range from 15 to 24 kcal/oz. Most infants can overcome a lower-density milk by consuming a greater volume.

Iron deficiency is approximately twice as common in breastfed infants; up to 30 percent have iron deficiency anemia, and more than 60 percent of the anemic infants are also iron deficient at 12 months of age (Pisacane et al., 1995), although the etiology is unclear. The iron content of human milk is low: 0.5 mg/L compared with 10 to 12 mg/L in supplemented cow-milk formulas. The absorption rate, however, is considerably higher. Breastfed infants absorb up to 50 percent of consumed iron, compared with a 7- to 12-percent absorption rate for formula-fed infants (Fomon et al., 1993). The risk of iron deficiency increases after 4 months of age since most full-term infants are born with adequate iron stores to support hemoglobin synthesis through the first 4 months after birth.

There have been increasing reports of nutritional rickets in breastfed infants, particularly in northern climates (Kreiter et al., 2000). This is likely due to lack of sunlight exposure, which is increasingly common with the use of sunscreens and the tendency to cover infants for health or cultural reasons. Human milk, like cow milk, is very low in vitamin D, with average concentrations of 24 to 68 IU/L. Since infants consume less than 0.5 L of milk/day in the first months of life, breastfed infants have vitamin D intake well below the Adequate Intake of 200 IU/day. With sun exposure this is not likely to be a problem. However infants born to mothers with vitamin D deficiency are at increased risk for rickets, as are those who are not exposed to the sun. The American Academy of Pediatrics and the Canadian Paediatric Society recently recommended supplementing all breastfed infants with 200 IU of vitamin D by 2 months of age (AAP, 2003; Canadian Paediatric Society, 1998).

In addition to transplacental passage of environmental allergens and dietary antigens, it is possible that susceptible infants may be sensitized to such agents by exposure to maternal milk. Although dietary antigens have been recovered in human milk, and allergen-specific IgE antibodies have been demonstrated in cord blood (Fälth-Magnusson, 1995; Lilja et al, 1988), available evidence suggests little or no role for breastmilk-associated food antigens in the development of food allergy (Businco et al., 1983; Fälth-Magnusson, 1995; Fälth-Magnusson and Kjellman, 1987).

Breastfed infants can be exposed to environmental toxins (e.g., lead and polychlorinated biphenyls), legal and illegal drugs, and infectious pathogens that the mother may harbor (e.g., Human Immunodeficiency Virus [HIV]). A discussion of all of the potential environmental toxins, drugs, and infectious agents is beyond the scope of this chapter. However it is important to note the effect of increasing rates of HIV infection worldwide and the potential for human milk to be both a vector of transmission of the virus from mother to infant and to contain protective anti-infective factors that may decrease the risk of vertical transmission. These risks and benefits must be weighed against the potential risks of formula feeding, not the least of which is preparation of formula with water contaminated with infectious agents (Humphrey and Iliff, 2001; Mbori-Ngacha et al., 2001; WHO, 1992).

SUMMARY

This chapter affirms that breastfeeding is the standard by which all other infant-feeding methods should be judged. This position has been taken by numerous professional bodies and reflects the fact that human milk is species specific and thus uniquely suited for human infant nutrition. It must be recognized, however, that using a human-milk composition or breastfeeding performance standard presents both regulatory and research issues when assessing the addition of ingredients new to infant formulas.

From a research standpoint, clinical studies that assess the effects of new ingredients will be difficult to design because infants cannot be randomized to be formula fed or breastfed. Furthermore, there may be significant non-nutritional confounding variables between the groups, including, but not limited to, factors related to which mothers breastfeed. Finally, human-milk composition varies considerably among individuals and within individuals over time, while infant formula content remains constant.

The committee anticipates that manufacturers will wish to add both ingredients that are currently contained in human milk, but not in formulas (e.g., LC-PUFAs), and those not found in human milk (e.g., prebiotics) to enhance the performance of formulas to a level at or nearer to human milk. Thus a breastfed control group should be part of experimental designs to assess the addition of ingredients new to infant formulas in order to provide a performance standard.

From a regulatory standpoint, the effect of an ingredient new to infant formulas is usually driven by a manufacturer's desire to produce products that mimic the advantages of breastfeeding. This motivation implies that formula in its current state is inferior (e.g., relatively neurologically or immunologically less beneficial, although not necessarily unsafe) when compared with human milk. Thus the safety (and efficacy) of any addition of an ingredient new to infant formulas will need to be judged against two control groups: one fed the previous iteration of the formula without the added ingredient, and one breastfed.

REFERENCES

AAFP (American Academy of Family Physicians). 2003. *Breastfeeding (Position Paper)*. Online. Available at http://www.aafp.org/x6633.xml?printxml. Accessed February 5, 2003.

AAP (American Academy of Pediatrics). 1997. Breast feeding and the use of human milk. *Pediatrics* 100:1035–1039.

AAP. 2003. Prevention of rickets and vitamin D deficiency: New guidelines for vitamin intake. *Pediatrics* 111:908–910.

Abt IA, ed. 1965. Allergy. In: *History of Pediatrics*. Philadelphia: W.B. Saunders. Pp. 259–260.

ADA (American Dietetic Association). 2001. Position of the American Dietetic Association: Breaking the barriers to breastfeeding. *J Am Diet Assoc* 101:1213–1220.

Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, Singer LT, Montalto MB, Jacobs JR, Qiu W, Bornstein MH. 2001. Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics* 108:372–381.

Barker DJP, Eriksson JG, Forsen T, Osmond C. 2002. Fetal origins of adult disease: Strength of effects and biological basis. *Int J Epidemiol* 31:1234–1239.

Beaudry M, Dufour R, Marcoux S. 1995. Relation between infant feeding and infections during the first six months of life. *J Pediatr* 126:191–197.

Benson JD, Masor ML. 1994. Infant formula development: Past, present and future. *Endocr Regul* 28:9–16.

Bergmann RL, Diepgen TL, Kuss O, Bergmann KE, Kujat J, Dudenhausen JW, Wahn U. 2002. Breastfeeding duration is a risk factor for atopic eczema. *Clin Exp Allergy* 32:205–209.

Bottcher MF, Jenmalm MC, Garofalo RP, Bjorksten B. 2000. Cytokines in breast milk from allergic and nonallergic mothers. *Pediatr Res* 47:157–162.

Brenner RR. 1974. The oxidative desaturation of unsaturated fatty acids in animals. *Mol Cell Biochem* 3:41–52.

Businco L, Marchetti F, Pellegrini G, Perlini R. 1983. Predictive value of cord blood IgE levels in "at risk" newborn babies and influence of type of feeding. *Clin Allergy* 13:503–508.

Butte NF, Wong WW, Ferlic L, Smith EO, Klein PD, Garza C. 1990. Energy expenditure and deposition of breast-fed and formula-fed infants during early infancy. *Pediatr Res* 28:631–640.

Canadian Paediatric Society, Dietitians of Canada and Health Canada. 1998. *Nutrition for Healthy Term Infants*. Ottawa: Minister of Public Works and Government Services.

Carlson SE, Werkman SH, Tolley EA. 1996. Effect of long-chain *n*-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr* 63:687–697.

Carver JD, Pimentel B, Cox WI, Barness LA. 1991. Dietary nucleotide effects upon immune function in infants. *Pediatrics* 88:359–363.

Cheng CM, Mervis RF, Niu SL, Salem N, Witters LA, Tseng V, Reinhardt R, Bondy CA. 2003. Insulin-like growth factor 1 is essential for normal dendritic growth. *J Neurosci Res* 73:1–9.

Coppa GV, Pierani P, Zampini L, Carloni I, Carlucci A, Gabrielli O. 1999. Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr Suppl* 430:89–94.

Dewey KG, Heinig MJ, Nommsen-Rivers LA. 1995. Differences in morbidity between breast-fed and formula-fed infants. *J Pediatr* 126:696–702.

Donovan SM, Odle J. 1994. Growth factors in milk as mediators of infant development. *Annu Rev Nutr* 14:147–167.

Duncan B, Schifman RB, Corrigan JJ Jr, Schaefer C. 1985. Iron and the exclusively breast-fed infant from birth to six months. *J Pediatr Gastroenterol Nutr* 4:421–425.

Duncan B, Ey J, Holberg CJ, Wright AL, Martinez FD, Taussig LM. 1993. Exclusive breast-feeding for at least 4 months protects against otitis media. *Pediatrics* 91:867–872.

Dvorak B, Fituch CC, Williams CS, Hurst NM, Schanler RJ. 2003. Increased epidermal growth factor levels in human milk of mothers with extremely premature infants. *Pediatr Res* 54:15–19.

Fälth-Magnusson K. 1995. Dietary restrictions during pregnancy. In: de Weck AL, Sampson HA, eds. *Intestinal Immunology and Food Allergy*. Nestlé Nutrition Workshop Series. Vol. 34. New York: Raven Press. Pp. 191–201.

Fälth-Magnusson K, Kjellman NI. 1987. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy—A randomized study. *J Allergy Clin Immunol* 80:868–875.

Fomon SJ. 1993. *Nutrition of Normal Infants*. St. Louis: Mosby-Year Book.

Fomon SJ. 2001. Infant feeding in the 20th century: Formula and Beikost. *J Nutr* 131:409S–420S.

Fomon SJ, Ziegler EE, Nelson SE. 1993. Erythrocyte incorporation of ingested ^{58}Fe by 56-day-old breast-fed and formula-fed infants. *Pediatr Res* 33:573–576.

Furukawa M, Narahara H, Yasuda K, Johnston JM. 1993. Presense of platelet-activating factor-acetylhydrolase in milk. *J Lipid Res* 34:1603–1609.

Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA. 2001. Risk of overweight among adolescents who were breastfed as infants. *J Am Med Assoc* 285:2461–2467.

Goldman AS, Chheda S, Garofalo R, Schmalstieg FC. 1996. Cytokines in human milk: Properties and potential effects upon the mammary gland and the neonate. *J Mammary Gland Biol Neoplasia* 1:251–258.

Grantham-McGregor SM, Fernald LC, Sethuraman K. 1999. Effects of health and nutrition on cognitive and behavioral development in children in the first three years of life. Part 1: Low birthweight, breastfeeding, and protein-energy malnutrition. *Food Nutr Bull* 20:53–75.

Halliwell B, Chirico S. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. *Am J Clin Nutr* 57:715S–725S.

Hamosh M. 1988. Fat needs for term and preterm infants. In: Tsang RC, Nichols BL, eds. *Nutrition During Infancy*. Philadelphia: Hanley and Belfus. Pp. 133–159.

Hanson LA, Carlsson B, Jalil F, Hahn-Zoric M, Hermodson S, Karlberg J, Mellander L, Khan SR, Lindblad B, Thiringer K, Zaman S. 1988. Antiviral and antibacterial factors in human milk. In: Hanson LA, ed. *Biology of Human Milk*. Nestlé Nutrition Workshop Series. Vol. 15. New York: Raven Press. Pp. 141–157.

Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B, Dewey KG. 1993. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: The DARLING Study. *Am J Clin Nutr* 58:152–161.

HHS/OWH (U.S. Department of Health and Human Services/Office on Women's Health). 2000. *HHS Blueprint for Action on Breastfeeding*. Washington, DC: DHHS.

Howell RR, Morriss FH, Pickering LK, eds. 1986. *Human Milk in Infant Nutrition and Health*. Springfield, IL: Charles C. Thomas.

Humphrey J, Iliff P. 2001. Is breast not best? Feeding babies born to HIV-positive mothers: Bringing balance to a complex issue. *Nutr Rev* 59:119–127.

Hynes MA, Buck LB, Gitt M, Barondes S, Dodd J, Jessell TM. 1989. Carbohydrate recognition in neuronal development: Structure and expression of surface oligosaccharides and beta-galactoside-binding lectins. *Ciba Found Symp* 145:189–210.

IOM (Institute of Medicine). 1991. *Nutrition During Lactation*. Washington, DC: National Academy Press.

Kling PJ. 2002. Roles of erythropoietin in human milk. *Acta Paediatr Suppl* 91:31–35.

Kreiter SR, Schwartz RP, Kirkman HN Jr, Charlton PA, Calikoglu AS, Davenport ML. 2000. Nutritional rickets in African American breast-fed infants. *J Pediatr* 137:153–157.

Kull I, Wickman M, Lilja G, Nordvall SL, Pershagen G. 2002. Breast feeding and allergic diseases in infants—A prospective birth cohort study. *Arch Dis Child* 87:478–481.

Lilja G, Dannaeus A, Fälth-Magnusson K, Graff-Lonnevig V, Johansson SGO, Kjellman N-IM, Öman H. 1988. Immune response of the atopic woman and foetus: Effects of high- and low-dose food allergen intake during pregnancy. *Clin Allergy* 18:131–142.

Lindmark-Mansson H, Akesson B. 2000. Antioxidative factors in milk. *Br J Nutr* 84:S103–S110.

Lonnerdal B, Hernell O. 1994. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. *Acta Paediatr* 83:367–373.

LSRO (Life Sciences Research Office). 1998. LSRO report: Assessment of nutrient requirements for infant formulas. *J Nutr* 128:2059S–2293S.

Lucas A, Cole TJ. 1990. Breast milk and neonatal necrotizing enterocolitis. *Lancet* 336:1519–1523.

Lucas A, Morley R, Cole TJ, Gore SM. 1994. A randomised multicentre study of human milk versus formula and later development in preterm infants. *Arch Dis Child* 70:F141–F146.

Lucas A, Morley R, Cole TJ. 1998. Randomised trial of early diet in preterm babies and later intelligence quotient. *Br Med J* 317:1481–1487.

MacLean WC Jr, Benson JD. 1989. Theory into practice: The incorporation of new knowledge into infant formula. *Semin Perinatol* 13:104–111.

Maheshwari A, Lu W, Lacson A, Barleycorn AA, Nolan S, Christensen RD, Calhoun DA. 2002. Effects of interleukin-8 on the developing human intestine. *Cytokine* 20:256–267.

Mbori-Ngacha D, Nduati R, John G, Reilly M, Richardson B, Mwatha A, Ndinya-Achola J, Bwayo J, Kreiss J. 2001. Morbidity and mortality in breastfed and formula-fed infants of HIV-1-infected women: A randomized clinical trial. *J Am Med Assoc* 286:2413–2420.

McCord JM. 1996. Effects of positive iron status at a cellular level. *Nutr Rev* 54:85–88.

Mestecky J, Blair C, Ogra PL, eds. 1990. Immunology of milk and the neonate. *Adv Exp Med Biol* 310:1–177.

Morrow-Tlucak M, Haude RH, Ernhart CB. 1988. Breastfeeding and cognitive development in the first 2 years of life. *Soc Sci Med* 26:635–639.

Mortensen EL, Michaelsen KF, Sanders SA, Reinisch JM. 2002. The association between duration of breastfeeding and adult intelligence. *J Am Med Assoc* 287:2365–2371.

Neeser JR, Chambaz A, Del Vedovo S, Prigent MJ, Guggenheim B. 1988. Specific and nonspecific inhibition of adhesion of oral actinomyces and streptococci to erythrocytes and polystyrene by caseinoglycopeptide derivatives. *Infect Immun* 56:3201–3208.

Nevinsky GA, Buneva VN. 2002. Human catalytic RNA- and DNA-hydrolyzing antibodies. *J Immunol Methods* 269:235–249.

Peterson JA, Scallan CD, Geriani RL, Hamosh M. 2001. Structural and functional aspects of three major glycoproteins of the human milk fat globule membrane. *Adv Exp Med Biol* 501:179–187.

Phaire T. 1955. In: Neale AV, Wallis HRE, eds. *The Boke of Chyldren.* Edinburgh, London: E & S Livingstone. P. 18.

Pisacane A, De Vizia B, Valiante A, Vaccaro F, Russo M, Grillo G, Giustardi A. 1995. Iron status in breast-fed infants. *J Pediatr* 127:429–431.

Popkin BM, Adair L, Akin JS, Black R, Briscoe J, Flieger W. 1990. Breast-feeding and diarrheal morbidity. *Pediatrics* 86:874–882.

Rivero-Urgell M, Santamaria-Orleans A. 2001. Oligosaccharides: Application in infant food. *Early Hum Dev* 65:S43–S52.

Ryan AS, Wenjun Z, Acosta A. 2002. Breastfeeding continues to increase into the new millennium. *Pediatrics* 110:1103–1109.

Saarinen UM, Kajosaari M. 1995. Breastfeeding as prophylaxis against atopic disease: Prospective follow-up study until 17 years old. *Lancet* 346:1065–1069.

Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R. 2002. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: A longitudinal study. *Lancet* 360:901–907.

Shu XO, Clemens J, Zheng W, Ying DM, Ji BT, Jin F. 1995. Infant breastfeeding and the risk of childhood lymphoma and leukaemia. *Int J Epidemiol* 24:27–32.

Singhal A, Farooqi IS, O'Rahilly S, Cole TJ, Fewtrell M, Lucas A. 2002. Early nutrition and leptin concentrations in later life. *Am J Clin Nutr* 75:993–999.

Uauy-Dagach R, Mena P. 1995. Nutritional role of omega-3 fatty acids during the perinatal period. *Clin Perinatol* 22:157–175.

Uauy RD, Birch DG, Birch EE, Tyson JE, Hoffman DR. 1990. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatr Res* 28:485–492.

Uauy R, Mena P, Wegher B, Nieto S, Salem N Jr. 2000. Long chain polyunsaturated fatty acid formation in neonates: Effect of gestational age and intrauterine growth. *Pediatr Res* 47:127–135.

van Zoeren-Grobben D, Lindeman JHN, Houdkamp E, Brand R, Schrijver J, Berger HM. 1994. Postnatal changes in plasma chain-breaking antioxidants in healthy preterm infants fed formula and/or human milk. *Am J Clin Nutr* 60:900–906.

Wallace JMW, Ferguson SJ, Loane P, Kell M, Millar S, Gillmore WS. 1997. Cytokines in human breast milk. *Br J Biomed Sci* 54:85–87.

Wang YS, Wu SY. 1996. The effect of exclusive breastfeeding on development and incidence of infection in infants. *J Hum Lact* 12:27–30.

WHO (World Health Organization). 1992. Consensus statement from the consultation on HIV transmission and breastfeeding. *J Hum Lact* 8:173–174.

WHO. 2002. *The Optimal Duration of Exclusive Breastfeeding. Report of an Expert Consultation.* Geneva: WHO.

Wright AL, Holberg CJ, Martinez FD, Morgan WJ, Taussig LM. 1989. Breast feeding and lower respiratory tract illness in the first year of life. Group Health Medical Associates. *Br Med J* 299:946–949.

Wroble M, Mash C, Williams L, McCall RB. 2002. Should long chain polyunsaturated fatty acids be added to infant formula to promote development? *Appl Dev Psychol* 23:99–112.

Xyni K, Rizos D, Giannaki G, Sarandakou S, Phocas I, Creatsas G. 2000. Soluble form of ICAM-1, VCAM-1, E- and L-selectin in human milk. *Mediators Inflamm* 9:133–140.

Zeisel SH, Char D, Sheard NF. 1986. Choline, phosphatidylcholine and sphingomyelin in human and bovine milk and infant formulas. *J Nutr* 116:50–58.

4

Strengthening the Current Processes to Evaluate New Ingredients for Infant Formulas

ABSTRACT

In the United States the Food and Drug Administration (FDA) is charged with monitoring the safety of infant formulas. If a manufacturer wishes to add a new ingredient to infant formulas, it must either file a Food Additive Petition or declare it a Generally Recognized as Safe (GRAS) substance through a GRAS Notification process. The GRAS Notification has become the route of choice for the introduction of new ingredients because it is scientifically rigorous, far more efficient, and equally or more transparent than the Food Additive Petition route. The committee found that the current regulatory system is limited by the following factors: (1) the complexity of the system has resulted in regulations that are not well understood by the scientific or medical community; (2) the system does not appear to adequately address the uniqueness of infancy and infant nutrition; (3) if a panel of experts is used to show consensus, there is a lack of guidelines for selecting a qualified and unbiased expert panel to evaluate the safety of the proposed new ingredient; and (4) formal guidelines for in-market surveillance do not exist for infant formulas.

The committee recommends that a set of guidelines be developed to provide a hierarchy of decision-making steps for manufacturers wishing to add new ingredients to infant formulas. In addition, elements of the safety assessments of infant formulas need to be standardized. Along with growth and development, other quality factors to be considered for these safety assessments are tolerance, allergenicity, impact of gastrointestinal flora, interference with bioavailability of other nutrients, and possible nutrient imbalances (if ratios or cofactors are important). To review the existing data and identify additional studies needed, the manufacturer should establish balanced, qualified expert panels in consultation with the regulatory agency. Also, the manufacturer should implement an appropriate in-market surveillance strategy that is based on findings from preclinical and clinical studies and the potential for harm to infants.

INTRODUCTION

Existing guidelines and regulations for evaluating the safety of conventional food ingredients (e.g., vitamins and minerals) added to infant formulas have been adequate in the past; however they were not designed to address the unique needs and vulnerabilities of infants and potential new types of ingredients. This chapter provides an overview of the current regulatory system with a discussion of the existing laws; definitions of safety, quality, food, drugs, and dietary supplements; and routes to add new ingredients to infant formulas in the United States. The committee then describes four major limitations of the current system. Finally, an approach is presented to assess the safety of new ingredients to be added to infant formulas, using the current GRAS Notification process as a starting model. The committee suggests that these guidelines could be applied in other countries as regulation parameters.

THE CURRENT U.S. REGULATORY SYSTEM

In the United States infant formulas and ingredients added to infant formulas fall within the purview of the Office of Food Additive Safety and the Office of Nutritional Products, Labeling and Dietary Supplements of FDA's Center for Food Safety and Applied Nutrition. The safety of new ingredients added to infant formulas is regulated under Section 409 of the Federal Food, Drug and Cosmetic (FD&C) Act (21 U.S.C. §348), which was the primary focus of the committee.

The Infant Formula Acts of 1980 (P.L. 96-359) and 1986 (P.L. 99-570) were incorporated into the FD&C Act as Section 412 (21 U.S.C §350a), which deals with proper manufacturing, formulation, and quality factors of infant formulas. This chapter will focus mainly on safety issues of ingredients (Section 409), but it should be noted that some aspects of Section 412 (quality factors) also play a critical role in the safety assessment process.

Foods, Dietary Supplements, and Drugs as Defined by the Federal Food, Drug and Cosmetic Act

The definition of foods, dietary supplements, and drugs and their respective safety standards are essential to the understanding of the regulatory differences between these classes of bioactive materials. It is beyond the scope of this chapter to delve into the rationale behind the regulations, but the concept of intended use is a critical differentiator between foods, dietary supplements, and drugs, which are defined in the following manner:

- *Foods* are "articles or components of articles used for food or drink for humans or animals" (21 U.S.C. §321 (f)(1)).
- *Drugs* are "articles intended for the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals" (21 U.S.C. §321 (g)(1)(b)).
- *Dietary Supplements* are products ". . . intended to supplement the diet that bear or contain one or more of the following dietary ingredients: a vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or a concentrate, metabolite, constituent, extract or combination of any ingredient described above" (21 U.S.C. §321 (ff)(2)).

Table 4-1 provides a comparison of the safety standards for foods, drugs, and dietary supplements. Infant formulas are considered food and, thus, a risk-benefit analysis would be inappropriate because foods are safe for everyone—young or old, male or female, healthy or

TABLE 4-1 Comparison of Safety Standards Included in Federal Regulations

Article	Safety Standard	Safety Decision
Foods or Food Additives (Federal Food, Drug and Cosmetic Act)	Reasonable certainty of no harm	Food Additive Petition—Food and Drug Administration (FDA) must approve that the material is safe Generally Recognized as Safe—Notifier makes safety determination; FDA reviews notification
Drugs (Federal Food, Drug and Cosmetic Act)	Risk-benefit	FDA must prove the material is safe and efficacious
Dietary Supplements (Dietary Supplement Health and Education Act)	Reasonable assurance that ingredient does not present a significant or unreasonable risk of illness or injury	FDA must prove the material is not safe

ill. Their purchase and consumption are unsupervised, unlike drugs for which access is carefully controlled and use is supervised by a physician. However because of the special role of infant formulas in the diet of infants, FDA requires that these products meet certain standards developed under the Infant Formula Act.

Quality Factors and Safety Aspects of Infant Formula

Section 412 of the FD&C Act and associated regulations issued by FDA deal with good manufacturing practices, quality control procedures, quality factors, notification requirements, and records and reports for the production of infant formulas. It is important to understand the concept of "quality factors" when considering the safety of ingredients, even though they are not considered as an integral part of the safety assessment of new substances under Section 409.

Quality factors are discussed in the report of the House Committee on Interstate and Foreign Commerce that accompanied the 1980 Infant Formula Act. An ingredient can only be used commercially (incorporated into infant formulas for sale) after the requirements of Section 412 (e.g., nutrient requirements and quality factors) are satisfied. This means that the new ingredient must be incorporated into the formula and the formula must then be tested for each required nutrient (i.e., protein, fat, essential fatty acids, vitamins, and minerals) under 21 U.S.C. 350a, Section 412(i). However the FD&C Act also mandates that the Secretary of the U.S. Department of Health of Human Services establish requirements for quality factors consistent with the scientific knowledge and states that the nutrient and nutrient level required by the Act may be revised. Under current regulations, the concept of quality factors has not been developed; the requirements focus on meeting the level of specific nutrients.

FDA has proposed to revise several aspects of its infant formula regulations, including requirements for quality factors and Good Manufacturing Practices (GMPs). FDA has proposed to revise "Quality Factors for Infant Formulas" (FDA, 1996), the subpart that defines the minimum quality factors for infant formulas. In these proposed regulations, FDA identifies two factors for which there is scientific basis to define them as quality factors, namely that the formula is "capable of supporting normal physical growth of infants" and "protein is of sufficient biological quality to meet the protein requirements of infants." FDA, in proposed rule Section 106.3(o), has defined quality factors in a manner that encompasses

several basic concepts, including bioavailability and healthy growth (FDA, 1996). The proposed rule states, "The quality factors, therefore provide a means of evaluating whether a nutrient has become less bioavailable than would be expected, so that it is not sufficiently effective to meet its normal nutritive functions, or whether its bioavailability has been enhanced to a level that raises safety concerns" (FDA, 1996, P. 36179). Furthermore, "FDA considers the concept of healthy growth to be broad, encompassing all aspects of physical growth and normal maturational development, including maturation of organ systems and achievement of normal functional development of motor, neurocognitive, and immune systems" (FDA, 1996, P. 36179). These parameters are also considered in the safety evaluation of any new ingredient (Section 409). Thus the safety aspects and the quality factors of the FD&C Act overlap in that they consider the safety of new ingredients by themselves (Section 409) and as part of the matrix (formula) (Section 412).

Routes to Add New Ingredients to Infant Formulas

Infant formulas are regulated as food. If a manufacturer wishes to add a new ingredient to an infant formula, it must follow one of three routes: it may determine a substance is GRAS without formally notifying FDA, it may file a GRAS Notification with FDA, or it may file a Food Additive Petition (see Box 4-1 for the definition of a food additive) with FDA. The GRAS Notification and the Food Additive Petition processes are described below.

GRAS Notification

GRAS status is based on common knowledge about the safety of the ingredient (the substance and its impurities) throughout the scientific community that is knowledgeable in food toxicology and related disciplines specific to the safety and intended use of the ingredient under consideration. A GRAS evaluation through scientific procedures is based on "generally available and accepted scientific data, information, methods, or principles, which ordinarily are published and may be corroborated by unpublished scientific data, information or methods" (FDA, 1997, P. 18960). There must be a "consensus among qualified experts about the safety of the substance for its intended use."

If the manufacturer believes the potential new ingredient is GRAS, the manufacturer will proceed to make the safety assessment and GRAS determination. Under the proposed GRAS Notification Rule (FDA, 1997), the manufacturer must declare that a substance is GRAS on the basis of scientific consensus by qualified experts. A manufacturer may convene a panel of

BOX 4-1 Definition of a Food Additive

". . . any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component of food or otherwise affecting the characteristics of any food . . . if such substance is not generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures* to be safe under conditions of its intended use."

**(or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food.)*

SOURCE: Federal Food, Drug and Cosmetic Act (21 U.S.C. §301).

experts. If the manufacturer concludes that the ingredient is safe for its intended use, the manufacturer notifies FDA. FDA reviews the notification (which includes a summary of the scientific evidence and historic use), and if FDA has no questions, it issues a letter of no objection. If FDA has questions concerning the safety of the ingredient, the manufacturer must satisfactorily answer them. In general, the quantity and quality of the data used to support a GRAS determination is comparable with the quantity and quality of the data used to support a Food Additive Petition.

Food Additive Petition

If a potential new ingredient cannot be determined to be GRAS, the manufacturer must file a petition proposing the issuance of a regulation prescribing the conditions under which the proposed additive may be safely used. The manufacturer supplies FDA with all pertinent data, especially safety data. The agency then conducts a comprehensive review of all the safety data and determines if the ingredient is safe for its intended use. In other words, FDA "owns" the safety decision after it makes an exhaustive, rigorous scientific evaluation of all appropriate safety studies. The process is generally lengthy because FDA has limited resources to review all the data and render a decision across a broad spectrum of ingredients and foods. This brief description of FDA's safety assessment process should not be interpreted to mean the process is simple; quite the contrary, the process is complex and rigorous.

Limitations of the Current Process

The GRAS Notification has become the route of choice for the introduction of new ingredients because it is scientifically rigorous, far more efficient, and equally or more transparent than the Food Additive Petition route. Still, the current process to assess the safety of ingredients new to infant formulas is complex and presents a number of limitations. The committee reviewed the current process and raised a number of issues that need to be addressed. Some are addressed here, some are addressed elsewhere in this report, and some need further study.

The regulatory scheme for the safety assessment of ingredients new to infant formulas is complex and spread out over several sections of the FD&C Act (Sections 409 and 412) and proposed regulations. In reality, the GRAS Notification is a scientifically rigorous and transparent determination of safety. It is flexible enough to accommodate the broad spectrum of issues, yet rigorous enough to ensure safety of the ingredient. However, for infant formulas, the complexity of the system has resulted in regulations that are not well understood by the scientific or medical community and misconceptions about the safety of GRAS ingredients for infant formulas may exist.

A second limitation of the current system is that it does not appear to adequately address the uniqueness of infancy and infant nutrition. Human milk or formulas are the sole source of nutrition for the first 4 to 6 months of life. Animal models to evaluate the safety of food ingredients in the period from birth to weaning are not addressed in FDA's *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food* (also known as the Redbook[1]; see Appendix C) (OFAS, 2001, 2003a). Conducting the

[1]This FDA document should not be confused with the American Academy of Pediatrics' Red Book of childhood infectious diseases.

types of toxicological studies that are discussed in the Redbook is a significant challenge (see Chapter 5).

Under current regulations, no measures of efficacy (i.e., health benefits) for the substance in question are required. Section 409 deals with safety of food additives independent of efficacy because efficacy has traditionally been considered a property of drugs and inappropriate for foods unless accompanied by an authorized health claim. Interestingly, many of the components of human milk have drug-like effects (e.g., prevention of disease) and are not considered classic nutrients.

The committee's primary charge was to assess safety of new ingredients added to infant formulas under U.S. regulations, with possible international applications. Safety and efficacy are clearly separated under U.S. regulations, but may not be so clearly delineated elsewhere. The committee was fully cognizant that efficacy is not a consideration of the GRAS process (Section 409) and focused its attention on matters related to safety as delineated in its charge. However safety and efficacy are not always mutually exclusive attributes and, in the case of infant formulas, which serve as a sole source of nutrition for a vulnerable population, there are overlaps of these parameters in Section 412. Although efficacy was outside the charge to the committee and an in-depth discussion was not attempted, the committee recommends consideration be given to convening a scientific expert committee to explore if benefit could be an appropriate requirement under Section 412.

A third limitation is the lack of guidelines for selecting a qualified and unbiased expert panel that may be used to evaluate the safety of the ingredient. The proposed GRAS regulations do not provide guidance about the panel selection or composition for manufacturers that use this mechanism to achieve consensus about the safety of the ingredient under consideration. Since only a few ingredients specifically intended for use in infant formulas have been evaluated by this route, manufacturers may be uncertain about selecting appropriate panel members until they have more experience with the process.

Nearly 25 percent of GRAS Notifications proposed since 1997 were rejected by FDA due to the inability of the notifier to satisfactorily answer FDA's questions regarding the substance and its health effects (OFAS, 2003b). The expert panel should have the appropriate experts to ask the right questions and form an opinion that is robust and of the highest scientific integrity. Guidelines for selecting a panel early in the process could improve the efficiency and objectivity of the process.

A fourth limitation is that formal guidelines for in-market surveillance do not exist for infant formulas. Infant formula manufacturers routinely conduct passive surveillance via toll-free calls, contact with health care professionals, and reports from their field sales force. Since infants are unable to communicate verbally, any adverse effects must be observed and reported through the parents or caregiver, thus special attention must be paid to detect adverse or unusual reactions. GRAS status is a time- and exposure-dependent judgment that requires the frequent monitoring of toll-free calls during the first 6 to 9 months after introduction of the product to the market. If the original safety determination was properly conducted, adverse outcomes should be rare and it should take a significant period of time to collect sufficient data in order to reaffirm GRAS status of the ingredient. A review of all pertinent data published and unpublished since the GRAS determination should be conducted approximately 2 to 4 years after introduction of the product. (Chapter 7 provides more details on current practices for in-market surveillance.)

In summary, the current GRAS review process was not designed specifically to address possible concerns for new ingredients in infant formulas or for situations where the sole intended use of the substance is in the only dietary product provided to an individual, as is the case for formula-fed infants.

PROPOSED SAFETY ASSESSMENT PROCESS

Use of a Hierarchical Approach

A hierarchical approach is recommended to assist in determining the appropriate level of assessment by considering: (1) the harm (e.g., toxicity), and (2) the potential adverse effects of a new ingredient. This hierarchical approach will guide the level of assessments to be applied to the new ingredient by considering the following factors:

- the reversibility of potential harmful effects,
- the severity and consequences of adverse effects,
- the time of onset of manifestations of the adverse effects,
- the likelihood that a new ingredient could adversely affect a specific system, and
- whether the effect would be common or rare.

This approach to evaluating the safety of new ingredients to be added to infant formulas was based on the uniqueness and vulnerability of the infant population. Therefore each step in the process requires empirical evidence from many disciplines and the application of the highest standards, whether using methods of bioassay, nutritional analysis, or basic chemistry. This approach is valuable in determining the relative importance of potential adverse effects for each specific new ingredient by providing generic templates for different steps in the safety assessment process rather than specific recommendations for each compound. It is neither realistic nor desirable to design individual templates for each new ingredient; rather expert panels can refine the generic templates as needed. This approach is designed for a broad spectrum of ingredients and could be applied to new ingredients to be added to infant formulas regardless of the regulatory process used.

The hierarchical approach is graphically presented in Figure 4-1 and by algorithms throughout the report. It is also applied in Appendix D to long-chain polyunsaturated fatty acids and probiotics. Each algorithm is a step-by-step decision tree that depicts the logic of the process, but it does not denote a particular chronology. For example, a manufacturer may initiate several different studies and procedures at the onset of the process, the results of which could be assessed at different steps in the algorithm. Any new ingredient considered for use in infant formulas must be considered in the context of its form, the matrix, and other ingredients with which it may interact.

RECOMMENDATION: Since infants have distinct needs and vulnerabilities, a set of guidelines should be developed to provide a hierarchy of decision-making steps for manufacturers seeking to add new ingredients to infant formulas. Because specific safety assessments will need to be targeted according to the nature of the ingredient, the set of guidelines should allow for flexibility in the approach, while being rigorous and scientifically based (see Figure 4-1).

Proposed Elements of the Safety Assessment

While each submission is unique and a reflection of the ingredient and its intended use, there is an opportunity to standardize the format and approach to the submission that would streamline its preparation and evaluation. The committee recommends amplifying the current approach without being prescriptive. It should be noted that some of the elements of the system proposed here are currently in place. The committee recognizes that some of its recommendations may require statutory changes.

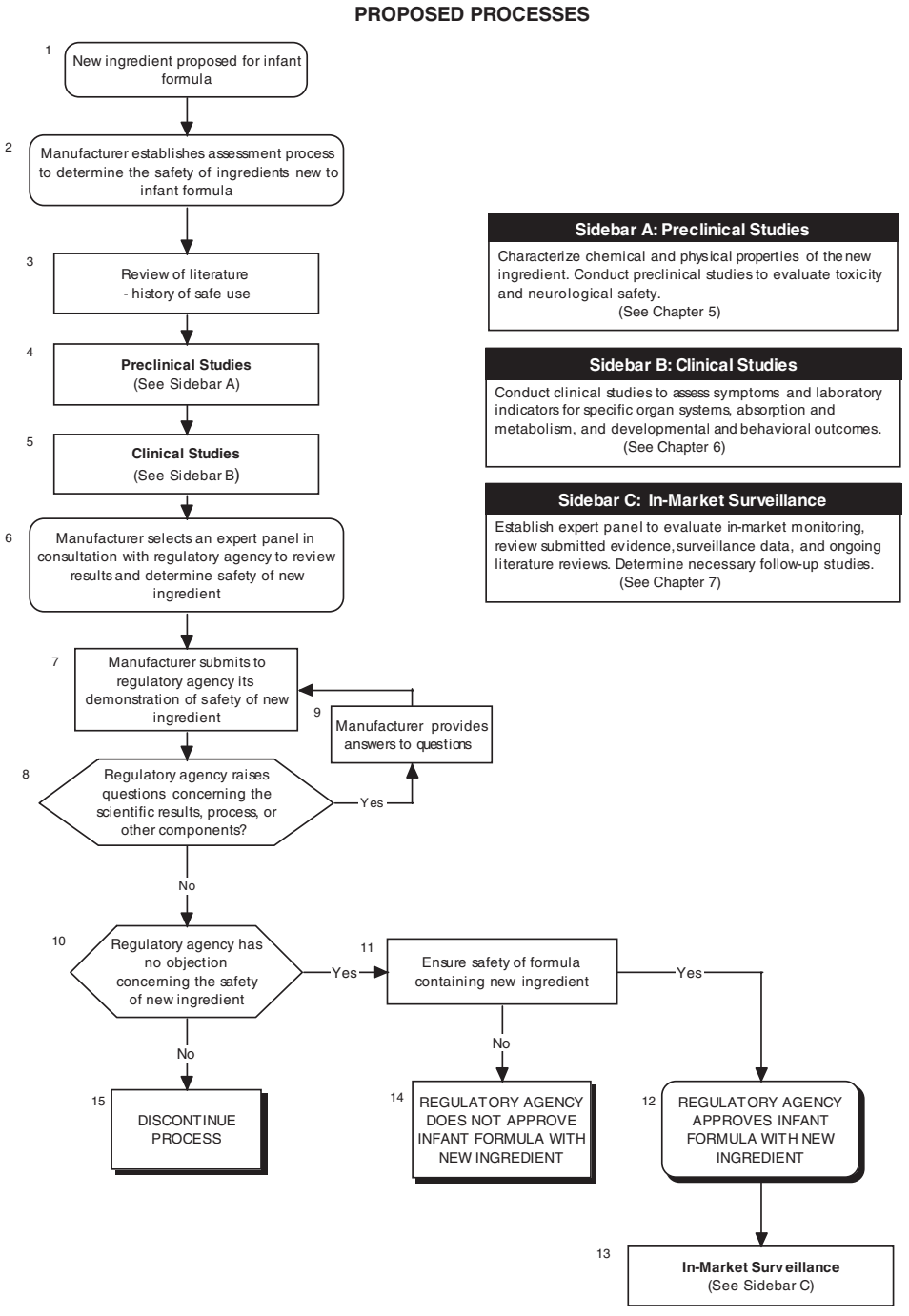

FIGURE 4-1 Proposed process for evaluating the safety of ingredients new to infant formulas algorithm. In-market assessment should be planned in conjunction with preclinical and clinical testing. This algorithm is modeled after the U.S. Generally Recognized as Safe Notification process; similar schemes can be adapted to other regulatory processes. [ ] = a state or condition, ◇ = a decision point, [ ] = an action, sidebar = an elaboration of recommendation or statement.

The committee proposes the following elements, described in detail below, for infant formula-related submissions to the regulatory agency:

1. Introduction
2. Background
3. Chemical or Biological Composition
4. Production Methods
5. Intended Use in Foods
6. Level of Exposure
7. Chemical Structure/Activity Relationships
8. Safety Assessment
9. Other Related Biological Activity or Interactions
10. Hazard Identification and Risk Assessment
11. Expert Panel Findings
12. General Availability of the Data

RECOMMENDATION: Elements of the safety assessments of infant formulas need to be standardized.

Element 1: Introduction

The introduction of a new ingredient submission should include the regulatory background to the submission and a summary of the submission.

Element 2: Background

The special nutritional requirements of infants and the role of formulas as a potential sole source of nutrients should be discussed in the context of the rationale for the addition of the proposed ingredient. A comparison with the composition of human milk should be provided as a reference point. While efficacy and risk-benefit should not be a consideration in the safety determination of an ingredient intended to be used in food, these factors cannot be totally ignored for biologically active substances, as they can be for the more traditional food ingredients (e.g., color, flavor, and preservatives). It must be recognized that it is unlikely that a manufacturer would add an ingredient solely because it is a component of human milk. Thus the rationale for the addition of the ingredient must be provided.

A targeted review of the literature and relevant commercial application within the United States or other countries could be helpful in understanding the novelty of the substance and its use (see Figure 4-1, Box 3). The extent and level of assessments will, in part, be decided upon by an expert panel's review of the history of use.

Element 3: Chemical or Biological Composition

Many of the substances that can be considered for addition to infant formulas are natural components of human milk, but their source is plants or animals, or they are synthesized. The safety issues therefore often revolve around the purity of the ingredients, as most are components of complex mixtures and may contain other biologically active or possibly toxic substances. The group of active components and the associated impurities must be well characterized (see Figure 4-1, Box 4). If the ingredient is produced synthetically, any by-products and residues must also be well characterized or removed.

Whether natural or synthetic, any complex ingredient must be examined to determine if its other components are toxic or in any way affect the function of the final product; byproducts shown to be of concern should be removed. If appropriate preclinical and clinical evaluations are conducted, this should lessen the safety concern. Sources of new ingredients must be determined to be free of dangerous impurities, such as pesticide residues, heavy metals, toxins, or pathogenic microorganisms. Allergenicity related to the ingredient or byproducts also should be addressed.

For new ingredients not present in human milk, a full review of safety will be required in the submission since there will likely be no history of safe use by infants. Reproducibility of the manufacturing process must be adequately demonstrated and tolerances established for the critical parameters. Complete specifications and analytical methods must be publicly available to allow independent validation of the characterization of the ingredient and associated tolerances. Stability of the ingredient during storage and any subsequent processing should be demonstrated.

Nontraditional sources, such as transgenic products and those produced by new methods, must have programs of evaluation designed to answer concerns related to these methods. For example, a review that includes adequate characterization and documentation of the genetic origins of the starting material and the characteristics of the process should be provided. The active components of the process (e.g., culture, recombinant deoxyribonucleic acid), along with the potential for introducing levels of undesirable compounds (e.g., toxins or allergens), should also be stated. As with other ingredients, the absence of substances that may present a hazard should be demonstrated.

Element 4: Production Methods

A detailed description of the methods used to manufacture the ingredient and the formula into which the ingredient is incorporated must be provided. Trade secret information should be clearly identified. Manufacturing and quality specifications should be established.

Most infant formulas are subjected to some form of heat treatment in order to provide a microbiologically safe product. These may include retorted or overpressure retorted, dried, aseptic, and pasteurized-refrigerated methods. New ingredients may require innovative processing methods to preserve the biological activity for the shelf life of the product. In some cases shelf life may be quite brief, refrigeration may be required, or separate components may need to be mixed at the time of feeding. Any changes in the processing and storage must be evaluated for safety and bioavailability. Individual ingredients have different rates of degradation (e.g., ascorbic acid degrades rapidly). The shelf life of the product depends on the ingredient with the most rapid degradation.

A wide variety of packaging (cans, bottles, composite canisters, pouches) is currently used for infant formulas, and current regulations define methods to assure safety and bioavailability. If new ingredients require novel package forms, the safety assessment must account for this.

GMPs (Good Manufacturing Practices) for infant formulas must also be addressed in this area of the submission to ensure that no unusual safety concerns arise.

Element 5: Intended Use in Foods

The technical, functional, or nutritional rationale for adding the substance to the infant formula should be clearly stated. If label claims will be made (e.g., “improves cognitive

performance," "lowers cholesterol"), the claim and references to support the claim should be cited. The intended use level should also be provided.

Element 6: Level of Exposure

The estimated daily intake of a substance in the context of the entire diet is of paramount importance in assessing the safety of any food ingredient. In the case of infant formulas, this determination should be straightforward as long as formulas are the sole source of nutrition. When solid food is introduced, a more detailed analysis must be conducted based on dietary records and panel data if the substance naturally occurs in the diet. For example, a careful record (diary) of all food consumed for a period of a few weeks should be kept and then analyzed for the substance in question. Another approach is to analyze available consumption data for foods containing the substance and calculating an estimated level of intake. These values are then combined with the estimated intake from the formula, appropriate safety factors are applied, and a safety determination is conducted.

Element 7: Chemical Structure/Activity Relationships

The analysis of the chemical structure/activity relationships is a useful approach to correlating molecular structure with biological activity or toxicity. In the absence of specific information about the toxicity of a compound, similarity in chemical structure to known toxicants can be used to estimate toxicity. Based on molecular structure, ingredients can be placed in one of the following categories (OFAS, 2001, 2003a):

- Category A – low potential for toxicity,
- Category B – intermediate potential for toxicity, or
- Category C – high potential for toxicity.

Categories, coupled with exposure levels, lead to the classification of ingredients into Concern Levels. Concern Levels are relative measures of the degree to which the use of an ingredient may present a hazard to human health. For example, an ingredient whose structure places it in Category C (high potential) and has a high level of exposure would result in assignment to Concern Level III (high concern), while an ingredient in Category A and low exposure would be placed in Concern Level I. The Concern Levels are used as a starting point to recommend toxicity tests, and the committee recommends using the same criteria for new ingredients added to infant formulas. Table 4-2 summarizes the toxicity tests recommended based on the Concern Levels.

Element 8: Safety Assessment

FDA's Redbook and the classic principles of toxicology are an excellent starting point for assessing the safety of new ingredients, but blindly applying the Redbook would be clearly inappropriate in the case of infant formulas. Many of the ingredients being considered for addition to infant formulas are naturally occurring nutrients in human milk, yet simply because an ingredient is a component of human milk does not mean it is safe. The matrix effect, ratios of other components, and interactions with other ingredients must be taken into account before a safety conclusion can be drawn for an infant formula. For

TABLE 4-2 Summary of Toxicity Tests Recommended for Different Levels of Concern of Food Ingredients

	Concern Levels		
Toxicity Tests[a]	I	II	III
Short-term tests for genetic toxicity	X	X	X
Metabolism and pharmacokinetic studies		X	X
Short-term toxicity tests with rodents	X[b]		
Subchronic toxicity tests with rodents		X[b]	X[b]
Subchronic toxicity tests with nonrodents		X[b]	
Reproduction study with teratology phase		X[b]	X[b]
One-year toxicity tests with nonrodents			X
Carcinogenicity study with rodents			X[c]
Chronic toxicity/carcinogenicity study with rodents			X[c,d]

[a]Not including dose range-finding studies, if appropriate.
[b]Including neurotoxicity and immunotoxicity screens.
[c]An in utero phase is recommended for one of the two recommended carcinogenicity studies with rodents, preferably the study with rats.
[d]Combined study may be performed as separate studies.
SOURCE: OFAS (2001).

ingredients that are already a part of human milk, the amount of toxicological testing required should be reduced, but the following must be thoroughly considered:

- What is the level of addition and interaction with other nutrients, processing, and storage?
- Are there any differences in absorption, distribution, metabolism, and excretion?
- Is the added ingredient chemically identical to the substance contained in human milk?
- Were contaminants introduced via the manufacturing process?
- Will the ingredient be safe for the shelf life of the product?

In addition to these considerations, the classic issues of subchronic toxicity, repeat-dose target organ toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and neurotoxicity should be considered and, where appropriate, in vitro and in vivo animal testing should be conducted (see Figure 4-1, Box 4). Animal models must be chosen appropriately to extrapolate results to humans (see Chapter 5). Dose, bioavailability, nutritional requirements, and developmental stage must be considered to prevent confounding the toxicology results.

Currently clinical trials for infant formulas with added new ingredients, although recommended, are not required by FDA, and the manufacturer decides when and if clinical trials are needed. The required tests focus on nutrient requirements, and only in cases of minor changes to the formulation can the manufacturer request a waiver of the tests requirements. However in the proposed rule (Section 106.3(o), subpart E, "Quality Factors for Infant Formulas"), quality factors are defined in terms of growth and development of the infant and therefore would require clinical trials as evidence of normal growth. The committee recommends that those clinical trials that address safety of the ingredient, metabolites, and matrix be included as part of the submission (see Figure 4-1, Box 5).

Further complicating the safety evaluation is the fact that most of the new ingredients are nutrients or biologically-active molecules that supposedly confer health benefits—not classic toxicants. In addition, some of the new ingredients are macronutrients, which require the application of different safety factors, experimental designs, and interpretation of results. In most cases micronutrients fed at the high doses typically used in toxicological testing would be toxic, thus safety factors of 10 or less are more appropriate than safety factors of 100.

Element 9: Other Related Biological Activity or Interactions

Early studies of the isolated ingredient may be helpful to identify biomarkers and to help in the design of more complex products. In addition to identifying any toxic or untoward reactions, preclinical studies may help identify appropriate levels of use. New ingredients should be evaluated in the matrix expected to be used in the final product in order to determine absorption, utilization, and activity when other ingredients are present. For example, soy formulas contain phytate, which can bind zinc, iron, and other divalent cations and make them unavailable. To allow for this, soy formulas are fortified with zinc, iron, and calcium. They also provide relatively large amounts of iron to allow for decreased absorption.

Ingredients are often available in more than one form. For example, selenium may be added to formulas as an inorganic salt or as an organic compound. Selection of the form most desirable for biological availability may present product-development challenges, but clear bioavailability is critical (MacLean and Benson, 1989). For infants, human milk and formulas are the sole source of nutrition for the first 4 to 6 months of life and an important source for the next 8 months. Although it is assumed that many new ingredients will be components of human milk or already accepted as safe for adults and children, the matrix in which the new ingredient is incorporated is very relevant in the safety evaluation (currently covered under Section 412). The importance of the matrix can be simply illustrated by the differences in cow-milk and soy-based formulas. Soy-based formulas may contain phytoestrogens, which could impact on the biological activity of a new ingredient. In addition to biological activity, factors in soy-based formulas also might impact bioavailability. For example, the fatty-acid profile of the matrix may differ, and this could impact on the metabolism of fatty acids added to provide a certain benefit. Since infants are a vulnerable population, the rationale for adding any new ingredient must be clearly stated so that qualified experts can make the safety evaluation with all factors known.

As these examples show, any new ingredient considered for use in infant formulas must be considered in the context of its form, the matrix, and other ingredients with which it may interact. Specific procedures for assuring bioavailability will depend on the compound under consideration.

RECOMMENDATION: Bioavailability is of special importance to infants and should be specifically addressed in any evaluation of the safety of infant formulas. Other factors that should be considered are: tolerance, allergenicity, impact of gastrointestinal flora, and possible nutrient imbalances (if ratios or cofactors are important).

Element 10: Hazard Identification and Risk Assessment

As stated previously, both the subject ingredient and associated contaminants or impurities may be hazardous to the target population, in this case term infants. The risk assessment for the ingredient versus the impurities are likely to be quite different. A no-observed-

adverse-effect level, safety factor, and estimated daily intake need to be determined, taking into account the differences in risk posed by the ingredient and possible contaminants. This will need to be determined for each new ingredient by each new expert panel.

Element 11: Expert Panel Findings

The findings of the expert panel are the heart of the safety evaluation submission. A properly constituted panel must be objective and consist of the appropriate types of scientific experts. In other words, the panel should have the appropriate experts to ask the right questions and form an opinion that is robust and of the highest scientific integrity. The manufacturer, in consultation with the regulatory agency, should determine an appropriate expert panel early in the process (see Figure 4-1, Box 6) to:

- guard against the theoretical possibility of bias,
- ensure an efficient process, and
- minimize the chance of rejection after submission.

The experts serving on the panel need to be free of any conflicts of interest (e.g., former employees or current stockholders of the manufacturer). Although there is no evidence of biased panels in the past, the potential for bias is a theoretical concern that needs to be addressed when selecting panel members.

The committee strongly recommends that in selecting appropriate experts to analyze ingredients new to infant formulas, the expert panel should include a physician with experience in clinical assessment, preferably a pediatrician. The composition of the rest of the panel should be determined in consultation with the regulatory agency and will depend on the nature of the ingredient (e.g., if dealing with probiotics, a microbiologist and immunologist should be included on the panel). A subpanel of specific experts may be needed in certain instances (e.g., when certain levels of in-market surveillance are needed; see Chapter 7). The panel members must be recognized experts in their field of expertise and highly regarded by their peers and the regulatory agency. They must conduct an independent critical evaluation of the information that is publicly available and conclude that they, as well as other experts in the field, would generally recognize that the ingredient is safe for its intended use.

The regulatory agency would review the panel's safety assessment and other data in the submission and if satisfied, would issue a letter of no objection. If the regulatory agency has questions and the manufacturer does not answer them satisfactorily, the agency can reject the submission, ask the company to withdraw it, or suggest consultation with additional experts qualified to opine on the specific safety concerns.

RECOMMENDATION: In seeking to add new ingredients to infant formulas, manufacturers should establish balanced, qualified expert panels in consultation with the regulatory agency. The panel members should review existing data and may identify a need for additional studies.

Element 12: General Availability of the Data

The requirement for all the pivotal data to be generally available for scrutiny by the scientific community at large makes the process transparent and robust. The data on which the safety determination is based must be published in peer-reviewed scientific journals and could be supplemented by secondary scientific literature, such as review articles and text-

books. For example, FDA's letter of no objection summarizing the basis for the GRAS Notification is made available on FDA's website (OFAS, 2003b), and the full notification should be available through the Freedom of Information Act. The responsibility for the safety determination rests with the company filing the notification, and it is the company's continuing responsibility to ensure that the food ingredients they market are safe and in compliance with all applicable legal and regulatory requirements.

SUMMARY

The safety assessment of infant formulas is complex and not fully standardized. While the current processes that regulate the addition of new ingredients to infant formulas are both flexible and scientifically rigorous, they do not adequately address the uniqueness of infants and infant nutrition. Also, they do not provide either enough guidelines on the selection of an appropriate expert panel that may be used to show consensus or guidelines for in-market surveillance. There is an opportunity to address these limitations and standardize the elements of the safety assessment of ingredients new to infant formulas without being overly prescriptive. The recommendations described here are meant to address the specific needs of infants in improving the regulatory process for this potentially vulnerable population group.

REFERENCES

FDA (Food and Drug Administration). 1996. Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula. Proposed rule. *Fed Regist* 61:36153–36219.

FDA. 1997. Substances Generally Recognized as Safe. Proposed rule. *Fed Regist* 62:18937–18964.

MacLean WC Jr, Benson JD. 1989. Theory into practice: The incorporation of new knowledge into infant formula. *Semin Perinatol* 13:104–111.

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS. 2003a. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

OFAS. 2003b. *Summary of all GRAS Notices*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~rdb/opa-gras.html. Accessed November 19, 2003.

5

Testing Ingredients with Preclinical Studies

ABSTRACT

Preclinical studies are a vital first step to assess the safety and quality of ingredients new to infant formulas. They must be performed before an ingredient can be considered for clinical studies in humans in order to determine the potential toxicity of the ingredient, its metabolites, and its matrix. Guidelines for these studies to assess the safety of infant formulas must be based on considerations of the diversity of potential new ingredients and the ingredients' source and matrix. In the United States, the Food and Drug Administration's (FDA) Redbook provides comprehensive guidelines for conducting preclinical studies to test the safety of food and color additives, but it often does not take the many special needs and vulnerabilities of infants into consideration. In Canada, there are no comprehensive guidelines for conducting preclinical studies, and decisions are made on a case-by-case basis using internationally accepted guidelines.

The committee recommends implementing a flexible, two-level assessment approach to help guide the expert panel's decisions on the appropriate preclinical studies (including preanimal tests; absorption, distribution, metabolism, and excretion studies; toxicity studies; and neurological studies) to assess the safety of ingredients new to infant formulas. Level 1 assessments include standard measures for each organ system required for all new ingredients (e.g., commonly used screening tests of cell and organ composition and function). Level 2 assessments include in-depth measures of organ systems that would be used to explicate equivocal level 1 findings or specific theoretical concerns not typically addressed by level 1 tests.

In addition to following established guidelines, a distinct set of procedures using appropriate cellular and animal models at relevant developmental stages should be included in studies to assess safety. The most commonly used animal models for general toxicological studies are the rat and mouse, but they are of limited use for developmental studies involving ingredients new to infant formulas because of the

difficulty of feeding formulas to a preweanling rodent. The nonhuman primate and the piglet are more amenable for these types of studies.

INTRODUCTION

This chapter describes the importance and the unique aspects of conducting preclinical studies to assess the safety of infant formulas. Current regulatory guidelines for preclinical studies are described, and a two-level assessment process is proposed. The committee's recommended two-level process is a flexible approach that can accommodate a variety of potential ingredients. For example, safety assessment might not be as intense for an ingredient that is not absorbed systemically. The manufacturer, in consultation with an expert panel, determines the types of tests conducted. The extent of this investigation will be determined on the basis of previous experience with the ingredient in other populations and on theoretical concerns based on the putative biological effects of the ingredient, its metabolite, and its matrix. Finally, the committee recommends the following preclinical tests described in detail below:

1. preanimal tests (including structure, stability, and solubility tests of the ingredients; genetic tests; and cellular studies)
2. absorption, distribution, metabolism, and excretion studies
3. toxicity studies (including acute, subchronic, chronic, developmental, and organ studies)
4. neurological studies

Neurological studies are described in a section separate from other toxicity studies because the scope of work defined for this project placed special emphasis on the potential effect of ingredients on the rapidly developing infant brain.

THE IMPORTANCE OF APPROPRIATE PRECLINICAL STUDIES

Preclinical studies must be performed before an ingredient can be considered for clinical studies in humans in order to determine the potential toxicity of the ingredient and its metabolites and their effects in the matrix. Preclinical studies allow researchers to expose cell cultures and experimental animals to doses of ingredients not normally encountered in human consumption. Results of such assessments are used to determine the threshold of toxicity for the given ingredient (i.e., the margin of safety).

Guidelines for preclinical studies to assess the safety of infant formulas must be based on considerations of:

- *The diversity of the potential new ingredients*. The new ingredients will possess different chemical characteristics, nutritional contributions, pharmacological activities, and physiological activities. The ingredients may be a conventional synthetic or an extracted single component, a plant extract, or a complex mixture. The ingredients may also be derived from novel sources or processes (e.g., products of fermentation or biotechnology). Such diversity requires preclinical guidelines that are clear but not overly prescriptive because of the disparity in the issues that each class of ingredient may represent. Nevertheless, given the vulnerability of the population to receive the ingredient (infants), it is incumbent upon the manufacturer, in consultation with the expert panel, to be overly conscientious in considering potential safety issues.

- *The ingredient's source and matrix*. The approach to evaluate ingredients new to infant formulas should be driven by the class of the functional substances *and* by the full characterization of the ingredient in the matrix of the infant formulas. In other words, the general approach is to deal with the targeted ingredient, as well as the nontargeted compounds, such as metabolites of the targeted ingredient, the vehicle required for delivery of the targeted ingredients, and impurities introduced in the manufacturing process.

CURRENT REGULATORY GUIDELINES FOR PRECLINICAL STUDIES

In considering how to construct guidelines for preclinical studies of ingredients new to infant formulas, the committee drew on existing guidelines for the testing of food ingredients and infant formulas. In the United States these are included in Sections 409 and 412 of the Federal Food Drug and Cosmetic (FD&C) Act (21 U.S.C. §301) and in the FDA Redbook (OFAS, 2001, 2003). The committee suggests that the risks of adding ingredients to sole nutrient sources, such as infant formulas, are not adequately addressed; however some aspects of the more stringent guidelines applied to new ingredients as articulated in the Redbook are appropriate for some, if not all, ingredients new to infant formulas.

Sections 409 and 412 of the Federal Food, Drug and Cosmetic Act

There are no explicit requirements for preclinical testing of infant formulas specified under Section 409 of the FD&C Act. The section stipulates that a petition to establish safety of a food additive shall contain "all relevant data bearing on the physical or other technical effect such additive is intended to produce. . ." (21 U.S.C. §301), but it does not dictate a specific type of preclinical study.

Under Section 412, which applies to infant formulas, a formula shall be deemed to be adulterated if it does not meet the quality factor requirements prescribed by the Secretary of Health under Subsection (b)(1). Subsection (b)(1) then states, "The secretary shall by regulation establish requirements for quality factors for infant formulas to the extent possible consistent with current scientific knowledge, including quality factor requirements for the nutrients required by subsection (i)." Currently, only protein quality is named as a quality factor in FDA regulations but there are no specific requirements to be met regarding quality factors. Subsection (i) specifies levels of certain nutrients (e.g., protein, fat, and specific vitamins) that are required to be met. There is no other requirement for any specific preclinical studies.

FDA Redbook

The FDA Redbook II and Redbook 2000 (OFAS, 2001, 2003) provide comprehensive guidelines for conducting preclinical studies to test the safety of food and color additives. Chapters IV and V in the Redbook II and 2000 describe:

- general guidelines for designing, conducting, and reporting results of toxicity studies,
- special considerations in toxicity studies (e.g., pathology, statistics), and
- guidelines for specific toxicity studies (e.g., genetic, acute, subchronic, chronic, immunotoxicity, and neurotoxicity).

These guidelines, however, do not consider the unique characteristics of having infants as consumers.

Canada's Food and Drug Regulations

There are no explicit requirements for preclinical testing of infant formulas specified under Canada's Food and Drug Regulations in Division 16 (Food Additives), Division 25 (Infant Formulas), or Division 28 (Novel Foods) (Canada, 2001). Divisions 16 and 28 require that a notification be submitted to Health Canada that includes information used to establish the safety of a food additive or a novel food. Health Canada refers manufacturers to internationally accepted guidelines for preclinical testing or asks to be consulted because decisions are made on a case-by-case basis.

OVERVIEW OF RECOMMENDED LEVELS OF ASSESSMENT

RECOMMENDATION: A hierarchy of two levels of preclinical assessment, using techniques from cellular-molecular studies through whole-animal studies, should be implemented to assess the safety of ingredients new to infant formulas on developing organ systems:

- **Level 1 assessments.** These are suggested standard measures for each organ system (e.g., gastrointestinal, blood, kidney, immune, endocrine, and brain) and are required for any new ingredient.
- **Level 2 assessments.** In-depth measures of organ systems or functions that would be performed to explain abnormalities found in level 1 assessments and specific theoretical concerns not typically addressed by level 1 tests. These are suggested measures to assess any new ingredient that primarily interacts with an organ system, has a metabolite that interacts with an organ system, or stimulates or changes the synthesis of factors (e.g., hormones, cytokines, immunoglobulins, endotoxin) that interact with an organ system.

Figure 5-1 describes the overall flow of proposed preclinical studies, from preanimal tests (Boxes 8 and 4) to toxicity tests (Box 4) to neurological tests (Box 7). Figure 5-2 describes the two assessment levels that should be considered when conducting preclinical studies, and it refers to tables in the text for specific measurements that could be conducted.

CONDUCTING PREANIMAL TESTS

Structure, Stability, and Solubility

Chemical and physical characterization is warranted if the targeted and nontargeted ingredients to be added to infant formulas are not well known and if there is no adequate literature about them to determine their safety. Thus the complete chemical structure and functional groups and the purity and stability of the intended and nontargeted ingredients present in the matrix must be determined using well-established physical methods (see Figure 5-1, Box 8). These methods include high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and thin layer chromatography (TLC). LC-MS is a particularly versatile technique. It can be used to assess the structure, molecular mass, and purity of most classes of compounds because derivatization is not necessary. The stability to temperature and ultraviolet light and the solubility properties of the ingredients should be established by standard methodology. The percentage of the unidentifiable materials should be stated in the notification or petition to the regulatory agency. The kind of

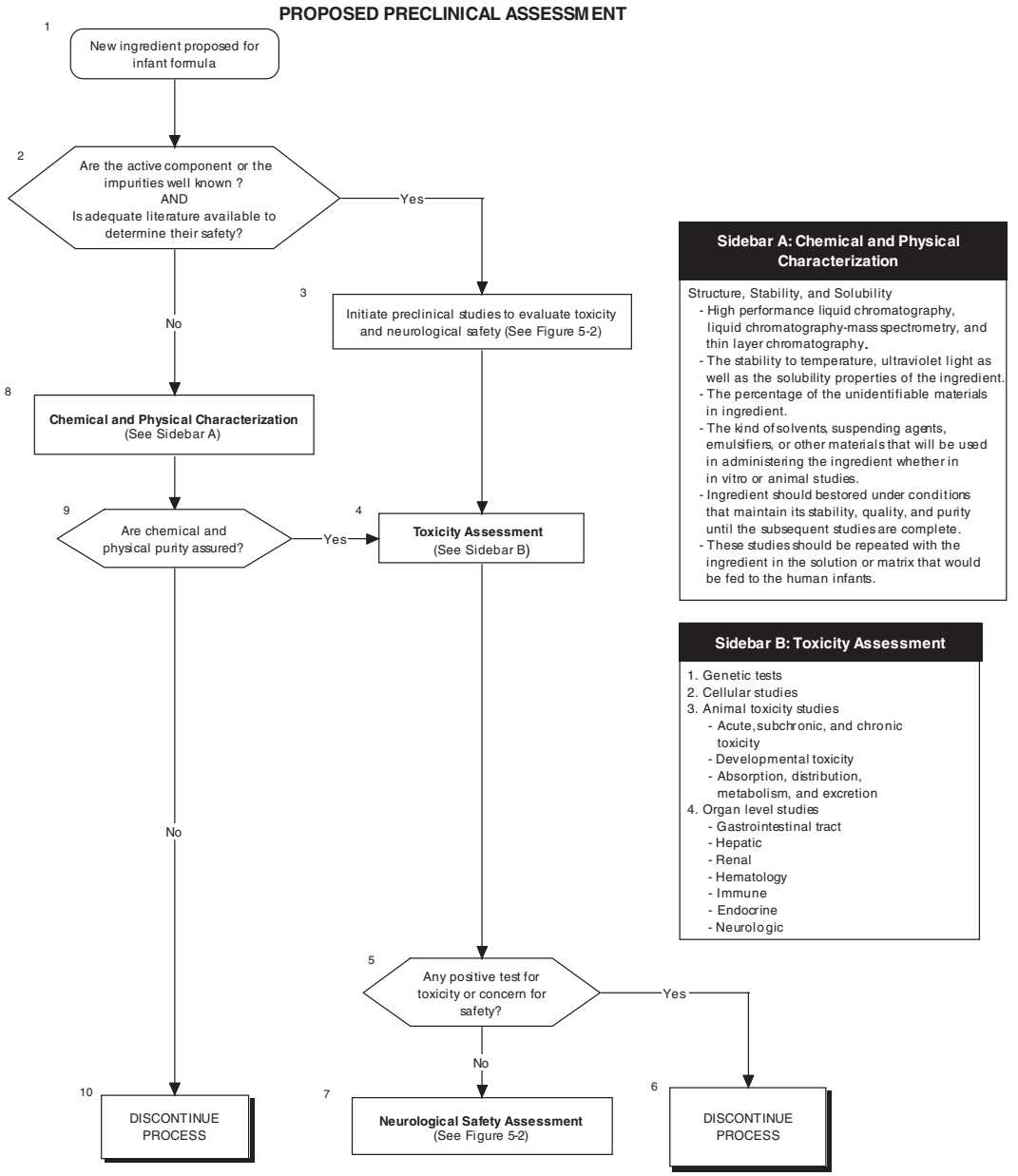

FIGURE 5-1 Proposed preclinical assessment algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

solvents, suspending agents, emulsifiers, or other material that will be used in administering the new ingredients during testing, whether using in vitro or animal studies, should be disclosed. The targeted ingredient should then be stored under conditions that maintain its stability, quality, and purity. The stability and solubility studies should be performed with the ingredient both in the solution and in the matrix that would be fed to human infants.

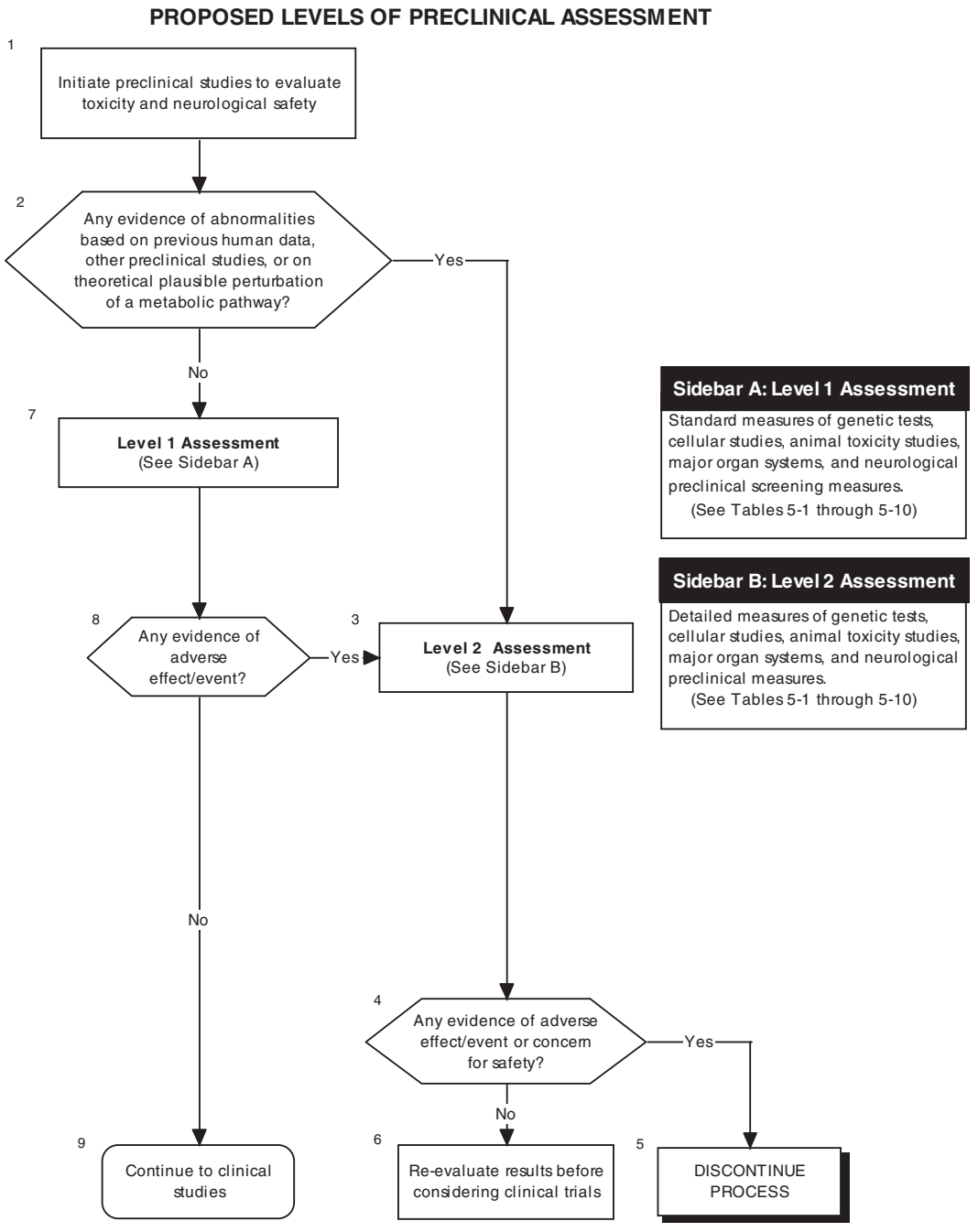

FIGURE 5-2 Proposed levels of preclinical assessment algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

Genetic Tests

Numerous genetic studies, such as those provided in the Redbook, are available, and the committee recommends evaluating all ingredients, their metabolites, or their secondary effectors for their ability, if any, to cause molecular changes in the deoxyribonucleic acid (DNA) or to cause structural changes in the chromosomes of cells (see Figure 5-1, Box 4). These changes may include forward and reverse mutations, point mutations, deletion mutations, chromosomal aberrations, micronuclei deletions, polymorphisms, DNA strand breaks, or unscheduled DNA synthesis. In vitro assessments use microorganisms and cells from multicellular animals; examples are listed in Table 5-1.

Cellular Studies

It is often most efficient to perform in vitro studies of metabolism before whole-animal (oral dosing) studies to provide information about future in vivo studies and estimate dosages to be used in preclinical animal studies. In vitro work and pharmacokinetic modeling can be used to predict the potential toxicity and in vivo kinetics of the ingredients and the matrix. The in vitro studies can also provide initial evidence of the level of clinical safety or risk.

Because in vitro studies are generally less complex than whole-animal studies, elucidation of an ingredient's metabolic pathway and toxicity characteristics may be facilitated. Different doses of the solubilized ingredient can be incubated with either confluent or preconfluent cells cultured in 10-cm dishes or 96-wells plates to establish uptake and toxicity levels of both the ingredient and its metabolic byproducts. Time-course and dose-response experiments should be conducted to check the growth characteristics of the cells and the toxicity of the ingredient. The production of metabolites can also be followed upon adding

TABLE 5-1 Examples of Potential in vitro Tests to Assess Genetic Toxicity

Test Name	Function of Test	Reference
Ames test	Microsome reverse mutation	Aeschbacher et al., 1983
Mouse lymphoma thymidine kinase gene mutation assay	Genetic forward mutations	McGregor et al., 1987, 1988a, 1988b; Myhr and Caspary, 1988; Myhr et al., 1990
Mammalian erythrocyte micronucleus test	Micronuclei deletions, chromosomal aberrations	Schlegel and MacGregor, 1982; Schlegel et al., 1986
Polymerase chain reaction	Changes in gene expression and deoxyribonucleic (DNA) sequence, polymorphisms, point mutations	Innis, 1990
DNA microarray[a]	Identifies genes that are up or down regulated	Cohen et al., 2002; Daniel, 2002; DeRisi and Iyer, 1999; Guengerich, 2001; Lee et al., 2000; Moreno-Aliaga et al., 2001; Perou et al., 1999; Wang, 2000; Williams, 1999
Proteonomics[a]	Identifies proteins that are altered after exposure to the ingredient	Anderson and Anderson, 1998; Bogyo and Hurley, 2003; Govorun and Archakov, 2002; Hochstrasser, 1998; Jungblut et al., 1999; MacBeath, 2002
Bioinformatics[a]	?	Vihinen, 2001

[a]These techniques are relatively new and are not standardized for clinical use.

the ingredient directly to the cellular extracts. In this case, different concentrations of the solubilized material are incubated with the cellular extract, and the production of metabolites as a function time is determined by analytical methods (e.g., HPLC, LC-MS, TLC). Radioactive and stable isotope studies are useful in tracing nutrient uptake by cells and intracellular metabolic pathways.

The in vitro systems can also be used to measure binding adduct and conjugate formation, transport across membranes, enzyme activity and concentration, enzyme substrate specificity, and other specific objectives. In addition to providing information on the effects of the ingredient and its metabolites on target receptors, cell-culture studies can also indicate subsequent downstream effects. In the future, DNA microarray technologies may be complemented by the capabilities of proteomics and metabolomics to assess the expression of expected and unexpected proteins and metabolites after ingredient exposure to the cells. However, at this point, these techniques are relatively new and are not standardized for clinical use. The principal problems that these advances confront lie in assessing the functional significance of the results of the analyses. Numerous algorithms in dealing with such analyses are available (Lander, 1999; Lee et al., 2000; Soukas et al., 2000) and should be consulted.

CONDUCTING ANIMAL TOXICITY STUDIES

Several toxicity studies must be performed in animals to ensure the safety of ingredients new to infant formulas (see Figure 5-1, Box 4). This section reviews the following issues:

- considerations when choosing animal models,
- general considerations when conducting animal studies, and
- considerations when conducting the following specific animal toxicity studies:
  - acute, subchronic, and chronic toxicity studies,
  - developmental toxicity studies,
  - absorption, distribution, metabolism, and excretion studies, and
  - organ-level studies.

RECOMMENDATION: In addition to established guidelines and regulations (e.g., the Redbook and regulations under Sections 409 and 412 of the FD&C Act), a distinct set of procedures using appropriate animal models at relevant developmental stages should be included in studies to assess safety.

Choosing Animal Models

Animal models are used as a tool in initial toxicology studies before human clinical trials are conducted. The end points of animal studies include:

- repeat dose toxicity (target organ evaluation),
- carcinogenicity (mutagenicity screen),
- developmental toxicity,
- reproductive toxicity, and
- neurotoxicity.

Animal models should be chosen appropriately, and it is especially important to look at the comparative development between the animal model and humans and to choose the

model that can be developmentally extrapolated to humans. For example, the animal model chosen must have digestive and metabolic similarities to the human infant and must be at the same point in development as the human infant who consumes formulas. Investigators should also ensure that the animal model and the reason for the studies have been justified such that there is an understanding of what can and cannot be extrapolated and how the preclinical data generated will be used to interpret the safety profile. All of the following questions must be answered before selecting animal models and moving ahead with a submission to the regulatory agency:

- What are the appropriate species?
- What are their ages?
- Should juvenile animals be assessed, or is age irrelevant for the animal models being considered?
- What safety factors are needed with these models, and what are going to be the relevant scientific disciplines?

At least two animal models should be selected, keeping in mind that the more animal models used, the better (OFAS, 2001, 2003), because converging lines of biological proof of cause and effect can be established. There are several additional factors to consider when conducting animal studies:

- The intended ingredient should be added to the animal's diet during the time in development that corresponds to when the average human infant will consume it.
- The bioavailability of the ingredient in the human infant must be known.
- The study must be designed to prevent differences in pharmacokinetics, handling of the ingredient, and dietary imbalance from competing ingredients.

The most commonly used animal models for general toxicological studies are the rat and mouse. However the rat and the mouse are of limited use for developmental studies involving ingredients new to infant formulas because of the difficulty of feeding formulas to a preweanling rodent. The nonhuman primate and the piglet are more amenable for these types of studies because they readily accept infant formulas as a nutrient source. The advantages and disadvantages of using each of these animal models is discussed below and summarized in Table 5-12.

Nonhuman Primate

Nonhuman primates are the closest analog to humans. Their diet is similar to humans and they can be fed infant formulas and followed developmentally. Humans and nonhuman primates are also comparable at a neural systems level. The animals display a wide array of sophisticated cognitive and motor behaviors (for review, see Bachevalier, 2001; Golub and Gershwin, 1984; Overman and Bachevalier, 2001). The nonhuman primate's neurodevelopmental trajectory is well described, with about a 4:1 ratio in relative time of development (e.g., 4 months of human brain development is equal to 1 month of development in the nonhuman primate).

Nonhuman primates can be more thoroughly assessed than humans through catheters in the blood stream, direct cerebrospinal fluid sampling, deep implanted electrodes, and repeated neuroimaging. These approaches allow a greater correlation of form and function changes.

Nonhuman primates have made substantial contributions to human nutrition studies, including assessment of the neurobiological effects of long-chain polyunsaturated fatty-acid (LC-PUFA) deficiency (Neuringer et al., 1986; Reisbick et al., 1994), iron deficiency (Kriete et al., 1995), and zinc deficiency (Golub et al., 1985). This model could be used to study changes in general behavior and speed of neural processing in response to the addition of ingredients with neurological effects, such as LC-PUFAs. Furthermore, neuroimaging with magnetic resonance imaging (MRI) may reveal differences in myelination or regional brain volumes.

The main disadvantages of using nonhuman primates are that they are very expensive to maintain, and most researchers are reluctant to sacrifice the animals to obtain brain tissue for a single analysis.

Piglet

Piglets are comparable in size to neonatal humans and metabolize fatty acids like humans do. Piglets can be raised on a relatively high-fat infant formulas and are an excellent choice for studies of hypoxia, endocrinology, and lipid metabolism. Piglets can be sacrificed and, thus, tissue can be obtained for analysis.

Pig models have been used extensively to study the role of added LC-PUFAs on the developing brain (Arbuckle et al., 1994; Craig-Schmidt et al., 1996; Hrboticky et al., 1990). These studies generally show that infant formulas enriched with LC-PUFAs and fed to neonatal pigs result in increased incorporation of these fatty acids into neuronal membranes and myelin. However piglets are difficult to work with in behavioral/learning paradigms, and neurodevelopmental form-function relationships are difficult to assess. Thus the inability to test these animals functionally to determine if the fatty acid incorporation results in beneficial or toxic effects is a significant drawback.

Rat

The rat is the most versatile model for toxicological testing for the following reasons:

- There is a wealth of information available on the rat model, including the nutrient effects on biochemistry, cell biology, neurophysiology, anatomy, and behavior.
- The developmental trajectory of the rat brain has been extensively compared with the human brain. For example, a postnatal day-7 rat has the structural maturity of a 34-week premature human, a postnatal day-12 animal is similar to a term human (Rice and Barone, 2000), and a postnatal day-28 rat approximates a human toddler.
- The rat's short reproductive cycle (21 days gestation) allows for rapid cycle studies and assessment of generational effects.
- Rats are excellent learners on basic cognitive (medial temporal lobe) tasks. Learning paradigms in rats have been correlated with human behavioral tests, allowing extrapolation of behavioral data between species (for review, see Overman and Bachevalier, 2001).

However the rat also has several limitations. Rats normally consume low-fat diets (approximately 5 percent fat), while human infants consume 45 to 55 percent of their calories as fat. Therefore the rat is a poor model for assessing the effects of altering or supplementing fats (including LC-PUFAs). Studies of LC-PUFA supplementation in the developing rat pup involved supplementing the mother and thus enriching the composition of rat milk (Haubner et

al., 2002; Pasquier et al., 2001). The matrix and source of these LC-PUFAs delivered to the rat pup were therefore different than the matrix and source of LC-PUFAs added to formulas and fed directly to the pup. Although a feeding system for artificial rat-milk substitute (formula) has been devised, it is invasive (i.e., it involves gastrostomy placement), it is difficult to maintain long term, and it is likely nonphysiological in nature.

Rats have been used extensively in brain efficacy and toxicity studies involving LC-PUFAs (Delion et al., 1994; Vazquez et al., 1994; Wainwright et al., 1997). For example, the GRAS Notification by Martek for the addition of LC-PUFAs to infant formulas lists multiple toxicity protocols involving rats (Hahn, 2000). Since it is difficult to feed infant formulas to a preweanling rat, the developmental tenets of timing, dose, and duration cannot be addressed. The literature cited in Martek's notification provides no mention of neurotoxicological effects in either the developing or the mature rat.

An appropriate approach to study the impact of LC-PUFAs on development would be to assess the animals for evidence of adequate synaptogenesis, myelin biochemistry, and myelin quantity. More specific probes for the effects of these ingredients on regulation of myelination (e.g., microtubule associated protein-2 or synaptophysin messenger ribonucleic acid) would represent targeted approaches to potential physiological processes likely to be affected by LC-PUFAs.

In some instances, such as when rats may possess different tissue metabolism and cortical function compared with humans, the rat model may be inappropriately extrapolated to humans. For example, the newborn human has relatively more cortical activity than the rat, so nutrient effects on cortical structures may be underestimated in the rat model. In contrast, the rat has a large and metabolically active hippocampus at birth relative to the human. Thus nutrient perturbations, such as iron deficiency, protein-energy malnutrition, or hypoglycemia, which profoundly affect the rat's hippocampus, may overestimate the effect in humans.

Mouse

The mouse is an excellent model because of the ability to manipulate its genetics and to link alterations in genotype to metabolic phenotypes. The mouse genome sequence is almost complete, so it provides a major resource to link gene expression to disease. For example, the ability to manipulate the genome to alter the uptake and processing of nutrients provides valuable information on mechanistic insight. A particularly powerful emerging technology is the conditional knock-out or knock-in model where nutrient dependent genes can be altered in specific regions of the brain at specific times of development (Lee et al., 1999).

Mice have been widely used in LC-PUFA research with respect to regulation of genes of lipid metabolism. In these studies it has been demonstrated that LC-PUFAs are potent inhibitors of lipogenic gene expression (Clarke, 2001; Ntambi, 1999; Shimomura et al., 1998).

The main disadvantages of the mouse model are:

- The mouse, like the rat, consumes a low-fat diet and cannot be fed infant formulas, making developmental effects difficult to assess.
- The mouse has different tissue metabolism compared with humans, particularly with respect to adipose tissue and liver lipid metabolism.
- The mouse has a limited neurobehavioral repertoire compared with the rat, making it more difficult to detect subtle neurocognitive effects after nutrient manipulations.

General Considerations When Conducting Animal Studies

Animal studies should include consideration of bioavailability, nutritional requirements and limitations, metabolic parameters, and developmental stage. They must be conducted in accordance with recommendations for the care and use of laboratory animals. The source and strain of the animals should be justified and stated in the submission. Investigators should choose:

- healthy animals,
- animals that have never been exposed to any experimental procedure,
- male and female animals, and
- animals that are capable of completing the entire study.

Testing should be performed on suckling animals and should continue after weaning and acclimation. Depending on the test and objective, investigators should choose several animals per sex for each experimental and control group. The animals should be assigned to the experimental and control groups in a randomized manner to minimize bias.

The diets to be used in the study should also be justified and stated in the submission. If the ingredient is to be fed, it must meet the nutritional requirements of the species. The diet fed to the control groups should be equivalent in nutritional value to the diets of the dosed groups. Because the amount of food consumed by each animal in the study cannot be determined when more than one animal is housed in each cage, it is recommended that the test animals be single-caged. Cross-fostering should be considered if a significant effect on feeding or nursing behavior is a potential effect of the added ingredient. For example, all studies of ingredients with potential neurological effects (e.g., LC-PUFAs, choline, oligosaccharides) should be evaluated in this manner. The bedding materials in the cages, ambient temperature, humidity, and lighting conditions also should be stated.

Acute, Subchronic, and Chronic Toxicity Studies

The FDA Redbook (OFAS, 2001, 2003) provides criteria for toxicity assessment of direct food additives and color additives used in food. These criteria are very stringent and it is debatable whether this degree of stringency is necessary for each ingredient new to infant formulas. Nevertheless the Redbook provides a scientifically conservative approach for the riskiest of new ingredients.

For all levels of toxicity studies, the route of administration of the supplement should approximate that of normal human exposure as closely as possible (e.g., through the diet in the case of infant formulas). The diets to be used in toxicological studies have to be properly selected. Natural-ingredient diets, as opposed to purified diets, are preferred because they do not usually affect nutrient balances. However each diet may have advantages and disadvantages that need to be considered.

If there is no information that can be used to determine the appropriate dose levels for short-term or subchronic toxicity levels, toxicity studies should begin with tests of acute toxicity, followed by subchronic, and finally chronic assessments. Table 5-2 provides guidelines for conducting these tests. The committee proposes the addition of developmental toxicity studies to the assessment of ingredients new to infant formulas in addition to the recommended guidance in the Redbook.

TABLE 5-2 Examples of Acute, Subchronic, Chronic, and Developmental Toxicity Studies

Study	Example
Acute toxicity	Single dose known to be toxic to the species, followed by observation of the animals for at least 2 weeks and establishment of the lethal dose for 50% of the animals (LD_{50}) for the ingredient, known bioactive metabolites, and biomass (source)
Subchronic toxicity	Generally conducted for 90 days in rats using doses established with the acute toxicity studies
Chronic toxicity	Can follow the subchronic and are usually carried out beyond the 90-day period and perhaps to adulthood
Developmental toxicity	Multigenerational study endpoints: generation toxicity F_0 (parental generation) and F_1 (second generation) Reproductive toxicity study endpoints: fertility, live born, weaning, viability indices, and male reproductive indices (e.g., testicular spermatid numbers)

SOURCE: OFAS (2001, 2003).

Developmental Toxicity Studies

The purpose of developmental toxicity studies is to evaluate the effects of the ingredient on developing fetuses that result from exposure of either parent prior to conception or to mothers during gestation. In the evaluation of ingredients new to infant formulas, the latter consideration is less relevant since pregnant mothers do not consume these ingredients. The main manifestations of an effect on the developing organism are death, structural abnormality, altered or retarded growth, and functional deficiency. Table 5-2 provides preclinical endpoints for developmental toxicity studies.

Absorption, Distribution, Metabolism, and Excretion Studies

The purpose of conducting absorption, distribution, metabolism, and excretion studies is to address the biological activity or availability of the ingredient when given to the infant. The toxicokinetics of the ingredient should be studied via the following:

- Absorption studies to assess the possible points of entry into the body (e.g., gastrointestinal tract, nose, mouth, and lung). Many, but not all (e.g., probiotics), new ingredients will be absorbed from the intestinal tract and have the potential to have important positive or negative biological effects. (Even probiotics can stimulate the synthesis of compounds that have important positive or negative biological effects.) It is incumbent on the manufacturer to assay for these effects, identify them, and assess their potential risk to the developing animal and human.
- Distribution studies to assess the subsequent transport and deposition throughout the body. Some tissues, such as bone, adipose, brain, kidney, liver, and hemotopeotic, may act as reservoirs for the new ingredient.
- Metabolism studies to assess the organ and cellular response to the presence of the ingredient. A relatively inert ingredient can be metabolized to a biologically potent compound that has extreme toxicity.
- Excretion studies to assess the removal of the ingredient from both systemic and nonsystemic stores. Organs of excretion include the liver, kidneys, gastrointestinal tract, lungs, and skin.

TABLE 5-3 Examples of Tests for Absorption, Distribution, Metabolism, and Excretion Assessment

Function	Assessment[a]
Absorption	Everted gut sacs and isolated intestinal loops, fecal material analysis, analysis of material in large intestine, radiolabel
Distribution	Whole body and organ autoradiography
Metabolism	Radioactive and stable isotopes, LC-MS, HPLC, TLC, DNA microarray,[b] metabolomics,[b] proteomics,[b] bioinformatics[b]
Excretion	Urine and other body fluid chemical analysis, LC-MS, HPLC, scintillation counting if ingredient is radioactive

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]LC-MS = liquid chromatography-mass spectrometry, HPLC = high performance liquid chromatography, TLC = thin layer chromatography, DNA = deoxyribonucleic acid.
[b]These techniques are relatively new and are not standardized for clinical use.

Unlike the organ system analyses described below, absorption, distribution, metabolism, and excretion studies are relatively uniform for any new ingredient and should follow the basic guidelines of the Redbook (OFAS, 2001, 2003). Table 5-3 provides examples of tests that could be conducted.

Organ-Level Studies

Examination of organs from the selected animal models can reveal important information concerning the effects of ingredients at the organ level. Different compounds will have different effects on the different organs and, as discussed earlier in this chapter, the general approach to evaluate the effect of new compounds added to infant formulas, as well as other material, should therefore be driven by the class of the functional substance and by the full characterization of the ingredient. The toxicity studies could be organ driven. All organs should be screened with their appropriate level 1 assessments, while more specific concerns with respect to a particular ingredient can be investigated through level 2 assessments (see Figure 5-2, Box 3).

All test animals should be subjected to complete gross necropsy, including examination of external surfaces, orifices, cranial cavity, carcass, and all organs, in the presence of a qualified pathologist. The ratio of organ weight to body weight should be documented. For a complete list of organs that should be examined and weighed, the reader should consult the Redbook (Chapter IV.B.1.) (OFAS, 2001). This list is extensive, but it is important because long-term adverse effects of the ingredient could later be found to be associated with organs that were not conspicuous or were ignored, and therefore unchecked at the time of the evaluation.

All organs should undergo a general screen or assessment, and then specific screens or assessments should be conducted based on expected biological effects of the new ingredient to be added to infant formulas. The evaluations of some of these key organs systems are detailed in the subsequent sections. Certain organs (e.g., liver, kidney, immune system, bone marrow, and brain) probably deserve greater scrutiny than others because of their functional or metabolic significance. For example, certain histological abnormalities, such as scattered focal mononuclear cell infiltrates in nonlymphoid tissues (e.g., liver and kidney), may indicate autoimmune disease. Also, if the ingredient is shown to either stimulate cell prolifera-

tion or to cause atrophy and cell depletion in any lymphoid organ, the effect is likely to be viewed as potentially immunotoxic and requires more definitive testing.

Gastrointestinal Tract Function

As Chapter 6 describes in detail, the development of the infant's gastrointestinal tract is essentially complete at birth and, therefore, assessments of its proper development will involve ensuring that its functions (e.g., digestion, absorption, secretion) have not been impaired by the addition of an ingredient new to infant formulas. Using a two-level approach for assessment, level 1 tests will include weighing the organ, performing histology examinations of the organ tissues, and other screening tests. Level 2 tests should be used to explicate equivocal level 1 findings or specific theoretical concerns not typically addressed by level 1 tests. Table 5-4 provides several examples of the types of tests that could be used in level 1 and level 2 assessments of the gastrointestinal tract.

Hepatic Function

The liver is involved in synthesis, metabolism, and excretion. Therefore, along with the above mentioned histology evaluation, tests that account for each of these functions must be performed as part of the level 1 assessment of liver health. Level 2 tests should be used to explicate equivocal level 1 findings or specific theoretical concerns not typically addressed by level 1 tests. Table 5-5 provides several examples of the types of tests that could be used in level 1 and level 2 assessments of liver health.

Renal Function

The kidney is the major excretory organ and also serves a role in blood pressure homeostasis. Ingredients new to infant formulas or their metabolites may be excreted by the kidney and may potentially damage the glomerular or reno-vascular function of the organ. Glomerular health is assessed by serum and urine chemical profiles in addition to the above mentioned histology. Abnormalities in level 1 assessments would lead to level 2 assessments, which should be tailored to the issues raised in level 1. Table 5-6 provides several examples of the types of tests that could be used in level 1 and level 2 assessments of kidney health.

TABLE 5-4 Gastrointestinal Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment
Level 1	Absorption
	Cell culture
	Organ weight/histology
Level 2	Isotopic absorption tests
	Microarray/proteonomics
	Receptor expression
	Specific histology stains
	Permeability tests

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.

TABLE 5-5 Hepatic Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment[a]
Level 1	Liver weight/histology, cell culture/mutagenicity
	Assessment of synthetic function: serum ALAT, ASAT, ornithine carbamyl transferase, albumin:globulin, coagulation profile, prothrombin time, partial thromboplastin time, radioactive amino acids, electrophoresis techniques for serum proteins
	Assessment of excretion function: gamma-glutyml transferase, LDH, bilirubin, alkaline phosphatase
	Assessment of metabolic function: total protein, albumin, fasting glucose, urea nitrogen, triglycerides (LDL, VLDL), cholesterol (HDL and LDL)
Level 2	Metabolism assessments
	Microarray/proteonomics
	Protein electrophoresis
	Special clotting factor levels
	Special imaging studies
	Special stains on histology

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]ALAT = alanine amino transferase, ASAT = aspartate amino transferase, LDH = lactate dehydrogenase, LDL = low-density lipoprotein, VLDL = very low-density lipoprotein, HDL = high-density lipoprotein.

Hematological Function

Ingredients new to infant formulas or their metabolites may have profound effects on the bone marrow. Numerous tests are available for level 1 assessments in addition to bone marrow histology. Abnormalities in level 1 assessments should lead to level 2 assessments of the relevant system that was perturbed by the addition of the new ingredient. Table 5-7 provides several examples of the types of tests that could be used in level 1 and level 2 assessments of hematological function.

Immunological Function

The immunological system is highly complex and has been shown to be sensitive to nutritional manipulation (Miles and Calder, 1998). The various effects of nutrients in the

TABLE 5-6 Renal Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment
Level 1	Cell culture/mutagenicity
	Kidney weight/histology
	Serum electrolytes, acid-base status, and blood urea nitrogen/creatinine
	Urinalysis
	Blood pressure
Level 2	Microarray/proteonomics
	Insulin clearance
	Special histology stains
	Special imaging studies

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.

TABLE 5-7 Hematology Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment
Level 1	Bone marrow histology
	Assessment of hematopoietic system: whole blood hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, total red cell count, red cell morphology
	Assessment of thrombopoietic system: platelet count, platelet morphology
	Assessment of white cells: total white cell count and the differential
	Assessment of the clotting system: prothrombin time, partial thromboplastin time, bleeding time
Level 2	Colony forming units
	Microarray/proteonomics
	Special bone marrow stains

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.

immunological system can be divided into those mediated by antigen-specific immunoglobulin (Ig) E (allergic reactions), other antibodies, T-cells, cytokines, and chemokines, and those mediated by nonimmunological mechanisms. The effects of nutrients, such as LC-PUFAs, on immune response have been extensively examined in laboratory animals and humans (for review, see Miles and Calder, 1998). One could argue that the value of immune system assessments for such nonproteinaceous food ingredients may be questionable since they are unlikely to affect the system or to provoke an allergic reaction. However any residue present in the matrix of an ingredient may be of protein nature and may cause an allergic reaction. Therefore even when assessing nonproteinaceous material, an assessment for allergenicity may be needed depending on the presence and source of impurities.

In addition to histopathological evaluation of the lymphoid tissues, level 1 assessments should include in vivo and in vitro tests with animal models that examine unusual incidences of maternal infections in order to evaluate potential developmental indicators of immunological toxicity (see Table 5-8).

As part of the assessment of the effect of a new ingredient on the immune system, the possibility of a new ingredient being an allergen (i.e., able to provoke an allergic reaction, mostly through IgE mediation) for an infant should also be evaluated. In the case of proteinaceous material with unknown allergenic properties, level 1 and level 2 assessments should be performed to assess their potential for allergenicity (see Table 5-8). A decision tree developed by Metcalfe and colleagues (1996), and later modified by a Food and Agriculture Organization of the United Nations/World Health Organization Expert Consultation (FAO/WHO, 2001), should serve as the basis for the level 1 assessment. In addition, the committee believes that further recommendations for in vitro evaluation of allergenicity by the Codex Ad Hoc Intergovernmental Task Force on Safety Assessment of Genetically Modified Foods (Codex Alimentarius Commission, 2003) should be followed.

It should be pointed out that development of allergic or immunologically mediated hypersensitivity relative to ingested foods represents a complex spectrum of symptomatology, which is also influenced by a large number of environmental and cultural factors (Sampson, 2002). Because the immune system of the infant is essentially complete, animal models that predict adult allergic responses can also serve as models for infants. Although some animal models, such as the brown Norway rat model, may provide a valuable tool in the future (Knippels and Penninks, 2003), appropriately validated animal models for assess-

TABLE 5-8 Immunology Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment[a]
Level 1	T-/B-cell quantitation and function (immunological analysis of B- and T-lymphocytes and T-lymphocytes subsets [Th+Ts or CD4 and CD8])
	Thymus, spleen, bone marrow, lymph nodes, tissue histology
	Electrophoresis (e.g., for changes in levels of γ-globulin fractions [IgG, IgM, IgA, IgE])
	Total serum complement and components of complement (e.g., C3 from CH_{50} determinations)
	Levels of prostoglandin E_2, balance of LTB_4 and LTB_5
	Immunochemical assays of γ-interferons and serum autoantibodies (antinuclear, antimitochondrial, antiparietal antibodies of B-lymphocytes)
	In vitro assays of activity of natural killer cells
	Mitogenic stimulation assay of B- and T-lymphocytes
	Macrophage activity assays
	Stem cell assays
	In vitro assays to assess allergenicity (source of the protein, amino acid sequence homology analysis, physicochemical properties)
Level 2	Microarray/proteonomics
	Special histology stains
	Stimulation tests of immune function using the T-dependent or independent antigens or human vaccines
	Cell-mediated immune reactivity and host-resistance assays

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]Th = T helper cells, Ts = T suppressor cells, CD4 = cell differentiation antigen 4, CD8 = cell differentiation antigen 8, IgG = immunoglobulin G, IgM = immunoglobulin M, IgA = immunoglobulin A, IgE = immunoglobulin E, LTB_4 = leukotriene B_4, LTB_5 = leukotriene B_5.

ment of human allergic disease are not currently available. The committee believes that it is of critical importance that animal models for allergenicity assessment in humans be developed. Until such models are validated, the assessment of allergic potential should be approached in vitro on a molecular level (e.g., utilizing sequence homologies, comparative digestive stabilities, functional antigenic determinants, and B- and/or T-cell epitope characteristics), and the nature of the demonstrated immune response to the known allergic products should be determined.

Endocrine Function

Growth abnormalities of the test animal is an important early indication of a possible effect of a new ingredient on the endocrine system. Because endocrine effects may not be immediately apparent in growth changes, nor in other metabolic functions, some screening tests are indicated. Table 5-9 provides several examples of the types of tests that could be used in level 1 and level 2 assessments of endocrine function.

CONDUCTING NEUROLOGICAL TESTS

Background

As explained in detail in Chapter 6, there are important reasons to include neurological tests in safety assessments of new ingredients for infant formulas, including the sensitivity of

TABLE 5-9 Endocrine Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment[a]
Level 1	Hormone levels (e.g., T4, TSH, LH, FSH, GH, CCK, NPY, cortisol, leptin), blood sugar, insulin
	Organ weight/histology (e.g., adrenals, ovaries, testes, pancreas, thyroid)
Level 2	Microarray/proteonomics
	Provocative endocrine tests (e.g., cosyntropin stimulation)
	Special histology stains

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]T4 = thyroxine, TSH = thyrotropin, LH = luteinizing hormone, FSH = follicle-stimulating hormone, GH = growth hormone, CCK = cholecystokinin, NPY=neuropeptide Y.

growth and development to toxic substances and the long-term predictive value of behavioral measures. Therefore the scope of work defined for this project placed special emphasis on the potential effects of ingredients to be added to infant formulas on the rapidly developing infant brain. This section discusses the preclinical assessment tools and models that can be utilized to assess ingredients with either positive or negative neurological effects. The approach is decidedly developmental because the effects of added ingredients (or any environmental stressor) in the developing animal are highly dependent on the timing, dose, and duration of ingredient exposure (Kretchmer et al., 1996).

Traditionally, two metrics—composition and performance—are applied as infant formula manufacturers continue to refine their products to match the gold standard of breastfeeding (Benson and Masor, 1994; MacLean and Benson, 1989). With respect to the latter, the impact of infant formulas on neurodevelopment has come to the forefront. It is widely accepted that breastfed infants demonstrate more advanced neurodevelopment (Lucas et al., 1998; Morrow-Tlucak et al., 1988; Mortensen et al., 2002; Wang and Wu, 1996). One potential explanation is that ingredients found in human milk, but not in infant formulas (e.g., LC-PUFAs, nucleotides, growth factors, and oligosaccharides), may be responsible (DeLucchi et al., 1987; Innis, 1992; MacLean and Benson, 1989). As these compounds are identified and added to infant formulas, valid and stringent assessments of their impacts on the developing brain should be undertaken.

Nutrients and growth factors regulate brain development during prenatal and postnatal life. Late fetal and early neonatal life is a period of rapid brain growth and development in most mammals, including humans (Rice and Barone, 2000; for review, see Dobbing, 1990). There is a wealth of information on the critical events that take place in the brain's development in the early neonatal period. At this stage of development, cell migration is complete, but myelination, synaptogenesis, dendritic arborization and pruning, and apoptosis are highly active. The rapidly growing brain is more vulnerable to restriction or loss of nutrients when compared with the more mature, less actively growing brain in later childhood and adulthood. Arguably, the rapidly developing brain is also more amenable to repair following nutritional perturbations and may be more highly influenced by nutrient supplements (Kretchmer et al., 1996). Researchers have argued that the persistence of neurological abnormalities after repletion of a nutrient deficiency acquired early in life provides evidence that early neurological vulnerability outweighs potential central nervous system (CNS) plasticity in the developing human (Lozoff et al., 2000; Rao, 2000).

Nutrients may have variable effects on the developing brain. Nutrient deficiencies may produce negative effects or may have no effect at all depending on the stage of brain

development (for review, see Georgieff and Rao, 2001). Similarly, nutrient overabundance or supplementation may produce positive effects, negative effects, or no effects, and the magnitude of the effects can be regionally different. For example, iron deficiency in the young rat brain can cause severe energy and structural deficits in the hippocampus (de Ungria et al., 2000; Rao et al., 1999) and can cause abnormalities in dopamine metabolism without energy deficits in the striatum (Erikson et al., 2000). At an older age, the same degree of iron deficiency causes no structural deficits. Thus any nutrient's effect on the developing brain will be based on the timing, the dose, and the duration of the exposure.

All nutrients are important for neuronal cell growth and development, but manipulation of some nutrients appears to cause more effects than others. Protein, energy, iron, zinc, selenium, iodine, folate, vitamin A, choline, and LC-PUFAs are nutritional components that influence early brain development with measurable clinical effects in humans and animal models (Georgieff and Rao, 2001). A nutrient that promotes normal brain development at one time and concentration may be toxic at another time or concentration. Many nutrients, such as iron, are regulated within a very narrow range because deficiency and toxicity have profound effects in brain development.

The potential effects of nutrients on brain development will determine the types of preclinical testing that are needed. Effects can be neuroanatomical, that is, affecting neuronal cell division and cell growth (e.g., size, complexity, synaptogenesis, dendritic arborization). Furthermore, nutrients can affect developing non-neuronal cells, such as oligodendrocytes, astrocytes, and microglia, in turn influencing myelination, other nutrient delivery, and cell trafficking. Nutrients can affect neurochemistry through alterations of neurotransmitter and receptor expression. They can affect neurophysiology and neurometabolism with subsequent alterations of signal propagation.

The fundamental question that must be answered in the assessment of nutrient effects on neurodevelopment is whether nutritionally induced alterations in brain development result in changes in brain function, loosely defined as behavior. Specific considerations include:

- Is this effect transient (e.g., only during the time when the nutrient is supplemented or deficient) or are there long-term gains or losses beyond the time of deficiency?
- How close is the linkage for each nutrient and each time period of development?
- What accounts for the individual variability in behavioral outcome of nutritional-based alterations in brain development?
- Does a lack of behavioral effect in spite of a measurable biochemical effect imply plasticity or alternate circuit development?
- Are there genetic polymorphisms from a change in nutrient status that confer greater or lesser risk or benefit to the host?

Many of these questions can be answered experimentally before a nutrient is added to formulas, but a multilevel, integrated approach is required because of the limitations of human brain analysis (Nelson et al., 2002). The multiple levels of approach are shown in Table 5-10. The integrated approach (see Table 5-11) chosen should be complementary and obey the laws of timing, dose, and duration (Kretchmer et al., 1996). The animal models must be developmentally appropriate with respect to timing of brain events and must coincide with the likely time of nutrient supplementation or deficiency in the human. In other words, for brain-behavior associations to be relevant, the time at which a given nutrient alters brain developmental processes in the animal model should coincide with the time that the nutrient deficit or supplement occurs in the infant population.

TABLE 5-10 Neurological Assessment: Examples of Tests in Level 1 and Level 2

Level	Assessment
Level 1	Neuronal/glial cell culture
	Histological examination
	General behavior
Level 2	Electrophysiology (implanted/surface)
	Magnetic resonance imaging
	Magnetic resonance spectroscopy
	Neurotransmitter concentrations/receptor expression
	Targeted behavior assessments (e.g., learning paradigms)

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, determines which tests are required based on a thorough analysis of the potential effects of the new ingredient.

The nonhuman primate, pig, rat, and mouse are the traditional models for neurological research. Table 5-12 and the information provided earlier in this chapter describe the advantages and disadvantages to using each model for neurological research.

Preclinical studies should assess the neurological safety of the nutrients by measuring the physiological and pharmacological levels of the nutrients. The goal is to match structural and biochemical alterations to changes in function (e.g., behavior). It must be recognized, however, that there can be a dissociation between biochemical and cellular changes in the brain and neurodevelopment (defined as behavior).

Environmental effects on the developing brain have been historically divided into effects on the motor and nonmotor systems. The motor system is far better understood than the nonmotor system because it is more discretely localized in the CNS and has a longer history of investigation. Principles learned from the motor system serve as a model for the current approaches to experimentation in the nonmotor system. The nonmotor system includes cognitive and noncognitive behaviors. Within cognition, a number of behaviors can be assessed, including memory and emotion. Memory is further divided into declarative or recognition memory and implicit or procedural memory (Squire, 1992). The neural circuits that subserve these memory systems are quite distinct and are differentially vulnerable to nutrient insults. The difference in ontogeny of the memory systems has been extensively reviewed by Nelson (1995).

It should be noted that many of the neural systems have mixed components. For example, there are connections between structures underlying recognition memory (e.g., hippocampus) and emotion (e.g., amygdala). Thus, for example, it may be difficult to know from behavioral testing alone whether the enhanced ability to perform a recognition memory task following a nutritional supplement is due to direct effects on the hippocampus or through attentional and motivational effects mediated by the amygdala. Certain structures, such as the cerebellum and basal ganglia, are involved in both motor and cognitive events.

TABLE 5-11 Integrated Approach to the Assessment of Neurodevelopment

Neurological Function	System of Assessment
Animal behavior (fit to human)	Nonhuman primate, rat
Biochemistry (neurochemistry)	Human, animal model
Brain structure	Human, animal model
Human behavior	Human
Physiology, cellular/molecular	Animal model, cell culture

TABLE 5-12 Summary of Animal Models Used in Preclinical Studies

Animal	Advantages	Disadvantages
Chicken	Immunology	No relevance to human nutrition, genetics
Dog	Metabolism, immunology, organs	Behavior, genetics
Hamster	Lipid metabolism	Immunology, genetics
Mouse	Genetics, molecular analysis, mechanisms, organs	Learning paradigms, developmental
Nonhuman primate	Functional/behavioral relationship to humans, similar diet as humans, immunological studies, dermatological studies, renal function, kidney biopsy, invasive assessment	Basic biochemistry, histopathology, genetics; expense and ethical concerns for neural tests
Pig	Comparable size to neonatal humans, lipid biochemistry, organs	Learning paradigms, poor model for genetics
Rabbit	Biochemistry, immunology	No relevance to human metabolism
Rat	Cellular, molecular analysis, behavior, organs, physiological studies; brain development is similar to human infant	Higher-level learning, genetics, developmental

The optimal use of an integrated methods approach should allow assessment of nutrient effects on behavior, neuroanatomy, neurochemistry, and form-function relationships (e.g., time-locked combinations of methods, such as functional MRI). It is important to have assessments that tap similar brain areas across species (from human to rodent), allowing extrapolation of CNS effects at the tissue level (rodents) to species where tissue is unavailable (humans, nonhuman primates). Table 5-13 provides examples of the cross-referencing of behavioral tasks across three species.

Neurological Assessment Techniques

Overall there must be a systematic approach to this important aspect of safety effects of ingredients added to infant formulas. Preclinical assessment must include whole-animal work with specific attention to regional brain effects. Here, neuroimaging through histochemistry at autopsy and through MRI in the living animal provides information. Neurochemistry experiments should target plausible biological mechanisms, particularly as to how nutrients might affect neurotransmission. Electrophysiology, using either deep-implanted electrodes or scalp-surface electrodes, provides information about neuronal population functions (Bachevalier, 2001; Fuster and Alexander, 1971) and can be cross-referenced to the assessment of humans conducted by evoked or event-related potentials (see Chapter 6).

The following sections describe various techniques for using preclinical studies to assess the effect of ingredients new to infant formulas on the developing brain. As with the non-

TABLE 5-13 Examples of Cross-Species Developmental-Neural Assessment Tools

Measure	Infant	Monkey	Rat
A-not-B	X	X	
Black-white discrimination	X	X	X
Delayed nonmatch to sample test	X	X	X
Maze		X	X
Paired comparison	X	X	X
Object discrimination	X	X	X

neurological organs, the committee proposes a two-level hierarchy with respect to application of these techniques in the preclinical studies. Level 1 assessments are required of all proposed ingredients and are designed to survey the neurological system of the developing brain. Level 2 assessments are performed if the ingredient to be added could theoretically affect brain development through a biologically plausible mechanism or if level 1 assessments have previously demonstrated a safety concern for the brain (Table 5-10).

Neuronal Genetics/Mutagenicity

In the future, evaluations should include neuronal and glial-cell culture work to assess the effect of the added ingredient on genomics, metabolomics, and proteomics with the purpose of evaluating mutagenicity and further functional consequences. Genomic techniques (e.g., DNA microarray analysis) are new and promising techniques that, once refined, will likely have great utility in assessing whether there are inductions of desirable or undesirable genes after exposure to an ingredient. These methodologies are still limited because of difficulties in interpreting the functionality of observed changes in gene expression. The parallel fields of proteomics and metabolomics complement the genomics methods by assessing the production of expected and unexpected proteins after nutrient exposures. Furthermore, cell culture work can inform about nutrient effects on target receptors (for the nutrient or its metabolite) (Alcantara et al., 1994) and the subsequent downstream effect on signaling cascades in the neurons (Gietzen and Magrum, 2001; Magrum et al., 1999). An excellent overview of this type of approach can be found in the work of Gietzen (2000) on imbalanced amino acid diet effects on feeding behavior as mediated through the neurons of the anterior piriform cortex. Gietzen's work serves as a model system for assessing nutrient effects on signaling cascades in the relevant neuronal systems. As noted earlier in this chapter, virtually every new ingredient has the potential to advertently or inadvertently alter basic cellular processes. Therefore neuronal-cell culture techniques should be considered level 1 assessments.

Neuronal- and glial-cell culture techniques were not reported in the GRAS Notification for LC-PUFAs. These techniques may have identified unwanted effects of these ingredients on gene expression through microarray screening analysis. The effect of these added ingredients on the signaling cascade involved in long-term potentiation could have been assessed in hippocampal CA1 cell recordings.

Neuroanatomy

There are a number of techniques that assess neuroanatomy, ranging from direct histological assessment by light, confocal, and electron microscopy to neuroimaging (e.g., MRI). Histological assessment allows a direct view of the brain and should be used as a level 1 tool to assess cytological effects of all proposed ingredients. The approach is used in the pig and rodent models and can visualize general neuronal structure (e.g., myelin, synapses, dendritic arborization, neuronal number), effector proteins (e.g., transporters, receptors), apoptosis/cell death, and regulatory elements (e.g., iron regulatory proteins). The limitation of the approach is that it only shows structure, and alterations of function may not always follow structural changes.

MRI can be used in the developing human and all typical animal models to assess nutrient effects on total brain volume, regional volumes, myelination, and the visualization of some nutrients (for review, see Casey et al., 2001). In spite of great technical advances, this method is generally insensitive and assesses only relatively large differences in protein-

energy or fatty acid status. Therefore it has poor predictive value for future subtle disability of neurological development in humans. At this time, neuroimaging should be categorized as a level 2 assessment.

Neurochemistry

Neurochemistry can be assessed either directly in the tissue once it is harvested, in the tissue of the living brain through microdialysis catheters (Chen et al., 1995), or in vivo through MRI spectroscopy (Tkáč et al., 2003). The goal is to assess the potential adverse effect of ingredients on neurotransmitters and intermediate metabolism. The advent of MRI spectroscopy allows for the in vivo, real time, repeatable assessment of the chemistry of the developing brain. The technique uses proton, phosphorous, or 13-carbon tracer nuclear magnetic resonance, and it can be used in humans and in animals as small as mice. Its main limitation is the inability to target compounds prospectively; the compounds that can be assessed are dependent on the major peaks that are visualized on the spectra. If the nutritional effect is on compounds that are in a concentration that is too low to result in a major peak, the technique is not useful. This technique is innovative and as yet not standardized. Therefore it should be considered a level 2 assessment in the evaluation of ingredients likely to affect neurochemistry.

The measurement of nutrient effects on neurotransmitter systems is an important part of nutritional research on brain development. The response of neurotransmitters to changes in nutrient availability can be directly measured in the rodent (Chen et al., 1995) and in neuronal cell culture. It is important with the glutamine system that cell cultures contain both neurons and glia since there is an important shuttle of glutamate and glutamine that takes place. It is also important to assess compensatory feedback mechanisms when transmitter concentrations are affected by nutrients. In response to increased transmitter release, there can be alterations in receptor number and affinity and in presynaptic reuptake mechanisms. The effect of iron deficiency on dopamine provides an excellent example (Chen et al., 1995). The assessment of neurotransmitter systems will be a level 2 assessment for most ingredients. However many compounds being considered for addition to infant formulas (e.g., choline) are likely to have a significant impact on these systems and will require this type of assessment.

Neurophysiology

Nutrients have effects on the electrophysiology of neuronal populations. A fundamental question that must be addressed in any nutrient-additive experiment is the plausible biological mechanism by which neuronal output is changed, which could result in a behavioral change. Examples include changes in calcium gating through alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid and N-methyl-D-aspartate receptors in brain areas involved in learning, which could alter long-term potentiation and learning behavior (Magrum et al., 1999). If nutrient supplements have a putative biological effect on myelination, there should be demonstrable effects on the speed of processing of myelinated circuits (Birch et al., 1992; Uauy-Dagach and Mena, 1995). Indirect effects must be considered as well. For example, steroids have significant effects on hippocampal anatomy, physiology, and gene expression (for review, see Sapolsky, 1994). Therefore if a nutrient supplement presents a stress to the developing organism with increased output of corticosteroids or estrogens, secondary effects of these steroids on the hippocampus must be considered. Electrophysiological testing will typically be warranted as a level 2 assessment when there is a reasonable concern that a new ingredient will affect neuronal function.

Behavior

Ultimately the preclinical assessment must shed light on any positive or negative effects on behavior since it is the summation of the efferent expression of brain activity. An assessment of general behavior of the animal model should be performed with any added ingredient. This is part of standard animal care during experiments. Particular attention should be paid to changes in typical behaviors, such as activity level, feeding, and comfort seeking. In the case of suspected specific regional or neurotransmitter effects, a more comprehensive second level evaluation targeting the potential neuropathology is required. Examples include specific motor, cognitive, and behavioral paradigms that map on the brain systems at risk. With any behavioral assessment, cross-referenced behavioral assessments in the motor and cognitive domain are important during the period of nutrient delivery to assess acute effects. Just as importantly, down-stream, long-term effects must also be evaluated to assess whether the added nutrient has permanent or transient effects and whether there are any "sleeper" effects.

SUMMARY

Preclinical studies are a vital first step to assess the safety and quality of ingredients new to infant formulas. Regulatory guidelines for preclinical studies must be based on considerations of the diversity of the potential new ingredients and the ingredients' source and matrix. In the United States, the FDA Redbook provides comprehensive guidelines for conducting preclinical studies to test the safety of food and color additives, but it often does not take the many special needs and vulnerabilities of infants into consideration.

The committee recommends a two-level preclinical approach with respect to assessing the safety of ingredients new to infant formulas. The approach should take into consideration model systems that are appropriate for the developing infant. All proposed new ingredients require assessments using level 1 techniques (Tables 5-1 through 5-10) at the discretion of the expert panel. Any proposed new ingredient with expected effects on structure or function, either on a theoretical basis or as a result of level 1 tests, will require appropriately targeted level 2 evaluations (Tables 5-1 through 5-10).

REFERENCES

Aeschbacher HU, Finot PA, Wolleb U. 1983. Interactions of histidine-containing test substances and extraction methods with Ames mutagenicity test. *Mutat Res* 113:103–116.

Alcantara O, Obeid L, Hannun Y, Ponka P, Boldt DH. 1994. Regulation of protein kinase C (PKC) expression by iron: Effect of different iron compounds on PKC-beta and PKC-alpha gene expression and role of the 5'-flanking region of the PKC-beta gene in the response to ferric transferrin. *Blood* 84:3510–3517.

Anderson NL, Anderson NG. 1998. Proteome and proteomics: New technologies, new concepts and new words. *Electrophoresis* 19:1853–1861.

Arbuckle LD, MacKinnon MJ, Innis SM. 1994. Formula 18:2(*n*-6) and 18:3(*n*-3) content and ratio influence long-chain polyunsaturated fatty acids in the developing piglet liver and central nervous system. *J Nutr* 124:289–298.

Bachevalier J. 2001. Neural bases of memory development: Insights from neuropsychological studies in non-human primates. In: Nelson CA, Luciana M, eds. *Handbook of Developmental Cognitive Neuroscience*. Cambridge, MA: MIT Press. Pp. 365–379.

Benson JD, Masor ML. 1994. Infant formula development: Past, present and future. *Endocr Regul* 28:9–16.

Birch DG, Birch EE, Hoffman DR, Uauy RD. 1992. Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci* 33:2365–2376.

Bogyo M, Hurley JH. 2003. Proteomics and genomics. *Curr Opin Chem Biol* 7:2–4.

Canada. 2001. *Departmental Consolidation of the Food and Drugs Act and the Food and Drug Regulations. (Food and Drugs Act)*. Ottawa: Minister of Public Works and Government Services Canada.

Casey BJ, Thomas KM, McCandliss B. 2001. Applications of magnetic resonance imaging to the study of development. In: Nelson CA, Luciana M, eds. *Handbook of Developmental Cognitive Neuroscience*. Cambridge, MA: MIT Press. Pp. 137–147.

Chen Q, Beard JL, Jones BC. 1995. Abnormal rat brain monoamine metabolism in iron deficiency anemia. *J Nutr Biochem* 6:486–493.

Clarke SD. 2001. Polyunsaturated fatty acid regulation of gene transcription: A molecular mechanism to improve the metabolic syndrome. *J Nutr* 131:1129–1132.

Codex Alimentarius Commission. 2003. *Codex Principles and Guidelines on Foods Derived from Biotechnology*. Prepublication. Online. Food and Agriculture Organization, World Health Organization. Available at ftp://ftp.fao.org/codex/2003Prepublicationtexts/Codex_Texts_on_Biotechnology(E).pdf. Accessed January 12, 2004.

Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM. 2002. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 297:240–243.

Craig-Schmidt MC, Stieh KE, Lien EL. 1996. Retinal fatty acids of piglets fed docosahexaenoic and arachidonic acids from microbial sources. *Lipids* 31:53–59.

Daniel H. 2002. Genomics and proteomics: Importance for the future of nutrition research. *Br J Nutr* 87:S305–S311.

de Ungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. 2000. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res* 48:169–176.

Delion S, Chalon S, Herault J, Guilloteau D, Besnard JC, Durand G. 1994. Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. *J Nutr* 124:2466–2476.

DeLucchi C, Pita ML, Faus MJ, Molina JA, Uauy R, Gil A. 1987. Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants. *J Pediatr Gastroenterol Nutr* 6:568–574.

DeRisi JL, Iyer VR. 1999. Genomics and array technology. *Curr Opin Oncol* 11:76–79.

Dobbing J. 1990. Vulnerable periods in developing brain. In: Dobbing J, ed. *Brain, Behaviour, and Iron in the Infant Diet*. New York: Springer-Verlag. Pp. 1–25.

Erikson KM, Jones BC, Beard JL. 2000. Iron deficiency alters dopamine transporter functioning in rat striatum. *J Nutr* 130:2831–2837.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). 2001. *Evaluation of Allergenicity of Genetically Modified Foods*. Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. Rome: FAO.

Fuster JM, Alexander GE. 1971. Neuron activity related to short-term memory. *Science* 173:652–654.

Georgieff MK, Rao R. 2001. The role of nutrition in cognitive development. In: Nelson CA, Luciana M, eds. *Handbook of Developmental Cognitive Neuroscience*. Cambridge, MA: MIT Press. Pp. 491–504.

Gietzen DW. 2000. Amino acid recognition in the central nervous system. In: Berthoud HR, Seeley RJ, eds. *Neural and Metabolic Control of Macronutrient Intake*. New York: CRC Press. Pp. 339–357.

Gietzen DW, Magrum LJ. 2001. Molecular mechanisms in the brain involved in the anorexia of branched-chain amino acid deficiency. *J Nutr* 131:851S–855S.

Golub MS, Gershwin ME. 1984. Standardized neonatal assessment in the rhesus monkey. In: Nathanielsz PW, Parer JT, eds. *Research in Perinatal Medicine (I)*. Ithaca, NY: Perinatology Press. Pp. 55–86.

Golub MS, Gershwin ME, Hurley LS, Hendrickx AG, Saito WY. 1985. Studies of marginal zinc deprivation in rhesus monkeys: Infant behavior. *Am J Clin Nutr* 42:1229–1239.

Govorun VM, Archakov AI. 2002. Proteomic technologies in modern biomedical science. *Biochemistry* 67:1109–1123.

Guengerich FP. 2001. Functional genomics and proteomics applied to the study of nutritional metabolism. *Nut Rev* 59:259–263.

Hahn MJ. 2000. Letter to Linda Kahl on February 29, 2000. GRAS notification on behalf of Martek Biosciences Corporation.

Haubner LY, Stockard JE, Saste MD, Benford VJ, Phelps CP, Chen LT, Barness L, Wiener D, Carver JD. 2002. Maternal dietary docosahexanoic acid content affects the rat pup auditory system. *Brain Res Bull* 58:1–5.

Hochstrasser DF. 1998. Proteome in perspective. *Clin Chem Lab Med* 36:825–836.

Hrboticky N, MacKinnon MJ, Innis SM. 1990. Effect of a vegetable oil formula rich in linoleic acid on tissue fatty acid accretion in the brain, liver, plasma, and erythrocytes of infant piglets. *Am J Clin Nutr* 51:173–182.

Innis MA. 1990. *PCR Protocols: A Guide to Methods and Applications*. San Diego: Academic Press.

Innis SM. 1992. Human milk and formula fatty acids. *J Pediatr* 120:S56–S61.

Jungblut PR, Zimny-Arndt U, Zeindl-Eberhart E, Stulik J, Koupilova K, Pleissner KP, Otto A, Muller EC, Sokolowska-Kohler W, Grabher G, Stoffler G. 1999. Proteomics in human disease: Cancer, heart and infectious diseases. *Electrophoresis* 20:2100–2110.

Knippels LMJ, Penninks AH. 2003. Assessment of the allergic potential of food protein extracts and proteins on oral application using the brown Norway rat model. *Environ Health Perspect* 111:233–238.

Kretchmer N, Beard JL, Carlson S. 1996. The role of nutrition in the development of normal cognition. *Am J Clin Nutr* 63:997S–1001S.

Kriete MF, Champoux M, Suomi SJ. 1995. Development of iron deficiency anemia in infant rhesus macaques. *Lab Anim Sci* 45:15–21.

Lander ES. 1999. Array of hope. *Nat Genet* 21:3–4.

Lee CK, Weindruch R, Prolla TA. 2000. Gene-expression profile of the ageing brain in mice. *Nat Genet* 25:294–297.

Lee KH, Calikoglu AS, Ye P, D'Ercole AJ. 1999. Insulin-like growth factor-I (IGF-I) ameliorates and IGF binding protein-1 (IGFBP-1) exacerbates the effects of undernutrition on brain growth during early postnatal life: Studies in IGF-I and IGFBP-1 transgenic mice. *Pediatr Res* 45:331–336.

Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. 2000. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics* 105:E51.

Lucas A, Morley R, Cole TJ. 1998. Randomised trial of early diet in preterm babies and later intelligence quotient. *Br Med J* 317:1481–1487.

MacBeath G. 2002. Protein microarrays and proteomics. *Nat Genet* 32:526–532.

MacLean WC Jr, Benson JD. 1989. Theory into practice: The incorporation of new knowledge into infant formula. *Semin Perinatol* 13:104–111.

Magrum LJ, Hickman MA, Gietzen DW. 1999. Increased intracellular calcium in rat anterior piriform cortex in response to threonine after threonine deprivation. *J Neurophysiol* 81:1147–1149.

McGregor DB, Martin R, Cattanach P, Edwards I, McBride D, Caspary WJ. 1987. Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay to coded chemicals. I: Results for nine compounds. *Environ Mutagen* 9:143–160.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Caspary WJ. 1988a. Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay to coded chemicals. II: 18 coded chemicals. *Environ Mol Mutagen* 11:91–118.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ. 1988b. Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay to coded chemicals. III: 72 coded chemicals. *Environ Mol Mutagen* 12:85–154.

Metcalfe DD, Astwood JD, Townsend R, Sampson HA, Taylor SL, Fuchs RL. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Crit Rev Food Sci Nutr* 36:S165–S186.

Miles EA, Calder PC. 1998. Modulation of immune function by dietary fatty acids. *Proc Nutr Soc* 5:277–292.

Moreno-Aliaga MJ, Marti A, Garcia-Foncillas J, Alfredo Martinez J. 2001. DNA hybridization arrays: A powerful technology for nutritional and obesity research. *Br J Nutr* 86:119–122.

Morrow-Tlucak M, Haude RH, Ernhart CB. 1988. Breastfeeding and cognitive development in the first 2 years of life. *Soc Sci Med* 26:635–639.

Mortensen EL, Michaelsen KF, Sanders SA, Reinisch JM. 2002. The association between duration of breastfeeding and adult intelligence. *J Am Med Assoc* 287:2365–2371.

Myhr BC, Caspary WJ. 1988. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. *Environ Mol Mutagen* 12:103–194.

Myhr B, McGregor D, Bowers L, Riach C, Brown AG, Edwards I, McBride D, Martin R, Caspary WJ. 1990. L5178Y mouse lymphoma cell mutation assay results with 41 compounds. *Environ Mol Mutagen* 16:138–167.

Nelson CA. 1995. The ontogeny of human memory: A cognitive neuroscience perspective. *Devel Psychol* 31:723–738.

Nelson CA, Bloom FE, Cameron JL, Amaral D, Dahl RE, Pine D. 2002. An integrative, multidisciplinary approach to the study of brain-behavior relations in the context of typical and atypical development. *Dev Psychopathol* 14:499–520.

Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. 1986. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc Natl Acad Sci USA* 83:4021–4025.

Ntambi JM. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J Lipid Res* 40:1549–1558.

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS (Office of Food Additive Safety). 2003. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

Overman WH, Bachevalier J. 2001. Inferences about the functional development of neural systems in children via the application of animal tests of cognition. In: Nelson CA, Luciana M, eds. *Handbook of Developmental Cognitive Neuroscience*. Cambridge, MA: MIT Press. Pp. 109–124.

Pasquier E, Ratnayake WM, Wolff RL. 2001. Effects of delta5 polyunsaturated fatty acids of maritime pine (*Pinus pinaster*) seed oil on the fatty acid profile of the developing brain of rats. *Lipids* 36:567–574.

Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, Pergamenschikov A, Williams CF, Zhu SX, Lee JCF, Lashkari D, Shalon D, Brown PO, Botstein D. 1999. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci USA* 96:9212–9217.

Reisbick S, Neuringer M, Hasnain R, Connor WE. 1994. Home cage behavior of rhesus monkeys with long-term deficiency of omega-3 fatty acids. *Physiol Behav* 55:231–239.

Rao R GM. 2000. Early nutrition and brain development. In: Nelson C, ed. *The Minnesota Symposia on Child Psychology. The Effects of Adversity on Neurobehavioral Development*. Vol. 31. Mahway, NJ: Lawrence Erlbaum Associates. Pp. 1–30.

Rao R, de Ungria M, Sullivan D, Wu P, Wobken JD, Nelson CA, Georgieff MK. 1999. Perinatal brain iron deficiency increases the vulnerability of rat hippocampus to hypoxic ischemic insult. *J Nutr* 129:199–206.

Rice D, Barone S. 2000. Critical periods of vulnerability for the developing nervous system: Evidence from humans and animal models. *Environ Health Perspect* 108:511–533.

Sampson HA. 2002. Peanut allergy. *New Engl J Med* 346:1294–1299.

Sapolsky RM. 1994. The physiological relevance of glucocorticoid endangerment of the hippocampus. *Ann N Y Acad Sci* 746:294–304.

Schlegel R, MacGregor JT. 1982. The persistence of micronuclei in peripheral blood erythrocytes: Detection of chronic chromosome breakage in mice. *Mutat Res* 104:367–369.

Schlegel R, MacGregor JT, Everson RB. 1986. Assessment of cytogenetic damage by quantitation of micronuclei in human peripheral blood erythrocytes. *Cancer Res* 46:3717–3721.

Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD. 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* 273:35299–35306.

Soukas A, Cohen P, Socci ND, Friedman JM. 2000. Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev* 14:963–980.

Squire LR. 1992. Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. *J Cogn Neurosci* 4:232–243.

Tkáč I, Rao R, Georgieff MK, Gruetter R. 2003. Developmental and regional changes in the neurochemical profile of the rat brain determined by in vivo ^{1}H NMR spectroscopy. *Magn Reson Med* 50:24–32.

Uauy-Dagach R, Mena P. 1995. Nutritional role of omega-3 fatty acids during the perinatal period. *Clin Perinatol* 22:157–175.

Vazquez E, Herrero I, Miras-Portugal MT, Sanchez-Prieto J. 1994. Role of arachidonic acid in the facilitation of glutamate release from rat cerebrocortical synaptosomes independent of metabotropic glutamate receptor responses. *Neurosci Lett* 174:9–13.

Vihinen M. 2001. Bioinformatics in proteomics. *Biomol Eng* 18:241–248.

Wainwright PE, Xing HC, Mutsaers L, McCutcheon D, Kyle D. 1997. Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (*n*-6):(*n*-3) ratio and very high levels of docosahexaenoic acid. *J Nutr* 127:184–193.

Wang J. 2000. From DNA biosensors to gene chips. *Nucleic Acids Res* 28:3011–3016.

Wang YS, Wu SY. 1996. The effect of exclusive breastfeeding on development and incidence of infection in infants. *J Hum Lact* 12:27–30.

Williams KL. 1999. Genomes and proteomes: Towards a multidimensional view of biology. *Electrophoresis* 20:678–688.

6

Going Beyond Current Clinical Studies

ABSTRACT

Clinical studies are essential to ensure the safety of infant formulas and any systematic deviation from normal physical growth and development attributable to a new ingredient should be considered a safety threat. Growth studies, currently a centerpiece of clinical evaluation of infant formulas, should include precise and reliable measurements of weight and length velocity and head circumference. Appropriate measures of body composition also require assessment. Duration of follow-up measurements should at least cover the period when infant formula remains the sole source of nutrients in the diet of the infant. However the committee believes that growth studies are not sufficient on their own to assess ingredients new to infant formulas. Specific guidelines are needed to determine "normal" growth and to establish what represents a biologically meaningful difference among groups of infants consuming different formulas. Specific recommendations are needed to establish a level of difference that represents a safety concern.

Regulatory guidelines should ensure that infant outcomes encompass, as the Food and Drug Administration (FDA) has proposed, "all aspects of physical growth and normal maturational development." Any systematic differences in clinical outcomes that can be attributed to an ingredient new to infant formulas should be considered a safety concern that requires careful evaluation and, if needed, further clinical study to identify the pathway through which the infant has been affected. The committee recommends that a hierarchy of two levels of clinical assessment be implemented with regard to growth and organ systems. Level 1 assessments should include checking for signs of all adverse laboratory indicators of the major organ systems. Level 2 assessments should include in-depth measures of organ systems or functions that would be performed to explain abnormalities found in level 1 assessments or specific theoretical concerns not typically addressed by level 1 tests.

There are a number of reasons why it is equally important to include developmental-behavioral outcomes in future studies of the safety of ingredients new to infant formulas: the measures are sensitive to exposure to toxic substances, they can have long-term predictive value, and bidirectional brain-behavior links exist. Therefore, assessment of clinical endpoints should include measurement of infant sensory-motor, cognitive, affectual, and neural function with instruments that follow recommended criteria. The committee recommends that a hierarchy of three levels of clinical assessment be developed and implemented to determine what levels are appropriate to apply with regard to developmental-behavioral-neural outcomes. The levels of assessment are: level 1 assessments, including developmental screening measures; level 2 assessments, including in-depth measures of infant functions in major developmental areas (single assessment for each area with one instrument); and level 3 assessments, including in-depth measures of infant functions in major developmental areas (repeated assessment with multiple instruments).

The instruments used for these assessments should satisfy the following criteria: be age appropriate, have predictive value for long-term consequences, be adequately sensitive, have documented brain-behavior links, have cross-species generalizability, assess specific function, and be easy to administer. In addition, the committee considers that certain design features (e.g., adequate statistical power) are essential in all clinical studies.

INTRODUCTION

This chapter provides an overview of clinical studies and a brief overview of the current regulatory requirements for them. The first part of the chapter includes a rationale for clinical assessment of growth, specific recommendations on what should be measured, and guidelines for interpretation of results. In the second part, the committee describes more specific clinical endpoints in each of the organ systems likely to be affected by ingredients new to infant formulas. In the last part of the chapter considerable attention is paid to behavioral and developmental endpoints because of the young infant's heightened sensitivity to potentially toxic substances and the long-term consequences of such exposures.

THE IMPORTANCE OF CLINICAL STUDIES

While preclinical laboratory and animal studies have substantial value for identifying potential safety concerns, they are limited in their ability to predict what may happen in human infants. Clinical studies in human infants are needed for several reasons. First, extrapolation from animal studies may be limited by differences between animal and human structure, physiology, and development. Second, extrapolation from isolated tissue studies is limited by the inability of such models to assess functions in the context of whole organ systems where coordination and integration are the rule. For example, the digestion and absorption of nutrients requires coordination of numerous gastrointestinal functions. Third, there may be no available animal or tissue models to test specific functions. For example, it is not possible to use animal models to duplicate clinically relevant allergic reactions to foreign proteins, to determine the effects of a substance on acceptance or tolerance of an infant formula, or to test some of the higher cognitive functions found only in humans.

CURRENT REGULATORY GUIDELINES FOR CLINICAL STUDIES

Canada's Food and Drug Regulations

There are no specific requirements for clinical testing of infant formulas set out under Canada's Food and Drug Regulations in Division 16 (Food Additives), Division 25 (Infant Formula), or Division 28 (Novel Foods) (Canada, 2001). Division 25 of the Regulations requires that a premarket submission with respect to a new infant formula or an infant formula that has undergone a major change in composition, manufacturing, or packaging include the evidence relied on to establish that the infant formula is nutritionally adequate to promote acceptable growth and development in infants when consumed in accordance with the directions for use. Divisions 16 and 28 require that data be submitted to Health Canada that include information used to establish the safety of a food additive or a novel food, respectively. Health Canada refers manufacturers to internationally accepted guidelines for clinical testing or asks to be consulted because decisions are made on a case-by-case basis.

Sections 409 and 412 of the Federal Food, Drug and Cosmetic Act

There are no explicit requirements for clinical testing of infant formulas specified under Section 409 of the Food, Drug and Cosmetic (FD&C) Act. Section 409 stipulates that a petition to establish safety of a food additive shall contain "all relevant data bearing on the physical or other technical effect such additive is intended to produce . . . ," but it does not dictate a specific type of clinical study.

Current regulations for infant formulas under Section 412 of the FD&C Act do not define quality factor requirements, such as physical growth, but only describe required nutrient levels, without considering bioavailability. This gap is addressed in a proposed rule (FDA, 1996), where assessment of physical growth, using anthropometry, is proposed "as an integrative indicator of net overall nutritional quality of the formula." The proposed rule further states, "as the science evolves, FDA anticipates being able to progress beyond generalized, nonspecific indicators of overall nutritional intakes (e.g., measures of physical growth) to more specific and sensitive measures of biochemical and functional nutritional status" (FDA, 1996, P. 36181). Thus neither the current nor the proposed rules identify specific requirements for other clinical studies.

FDA Redbook

FDA does not require petitioners to conduct human clinical studies to support the safety of food additives or color additives used in food, but, if deemed necessary, it recommends that the studies conform to guidelines presented in section VI.A. of the Redbook (OFAS, 2001, 2003). These guidelines are comprehensive and relevant for the clinical testing of ingredients new to infant formulas.

General guidance is provided to identify the scientific and ethical principles for clinical studies, including the need for presentation of a defensible rationale for human studies. The Redbook states that this rationale should be based on:

- adequate preclinical investigations,
- results of clinical studies conducted elsewhere,
- consideration of the organs and organ systems that may be affected, and

• careful attention to the qualifications of investigators and the safety and ethical treatment of subjects in clinical trials.

The Redbook suggests the sequence of and subjects for clinical studies. Early clinical studies are to determine the "metabolism and level of the food or food additive that gives an adverse or toxic response in man" (specifically physiological studies of the additive's disposition, its potential to induce enzyme levels or increase activity, and its interactions with other nutrients) (OFAS, 2001, P. 183). In general children are to be excluded from these early (typically acute or shorter duration) clinical studies. However tolerance studies, which are to be included among early studies, need to be conducted in infants because of the special nature of infant formulas.

Infants are more likely to be included in what the Redbook describes as chronic intake studies, which are to be conducted once general safety in humans is established in the early adult studies. Here, the Redbook provides specific guidance on protocol design, study population, and statistical analyses, as well as on how reports of clinical studies should be presented. Box 6-1 lists questions that should be answered when conducting studies to determine the safety of a proposed additive.

GENERAL APPROACH TO CONDUCTING CLINICAL STUDIES

In the conceptualization of the range of infant health concerns, the committee was guided by the following: "FDA considers the concept of 'healthy growth' to be broad, encompassing all aspects of physical growth and normal maturational development, including maturation of organ systems and achievement of normal functional development of motor, neurocognitive, and immune systems. All of these growth and maturational developmental processes are major determinants of an infant's ability to achieve his/her biological potential, and all can be affected by the nutritional status of an infant" (FDA, 1996, P. 36179).

The committee proposes the use of a multilevel approach to establish more comprehensive guidelines to ensure that infant outcomes encompass "all aspects of physical growth and normal maturational development." Figure 6-1 illustrates the three different types of clinical studies recommended by the committee, including assessment of growth, organ systems, and development and behavior. Figures 6-2 and 6-3 further explain the clinical studies through the proposed two-level approach to organ systems and the three-level approach to development-

BOX 6-1 Questions That Should Be Answered When Conducting Clinical Studies

- How is the food or food additive absorbed, metabolized, deposited in tissue, and excreted?
- What is the half-life of the food or food additive in the human body?
- How may interactions between the food or food additive and nutrients or medications compromise the availability of any of these substances (including the consideration of the matrix)?
- How does the food or food additive affect the function of human organs and organ systems (including infant growth and development)?
- What are the possible adverse reactions to the food or food additive in the general population of individuals who are likely to use the substance and in special (more sensitive) populations?

SOURCE: OFAS (2001, 2003).

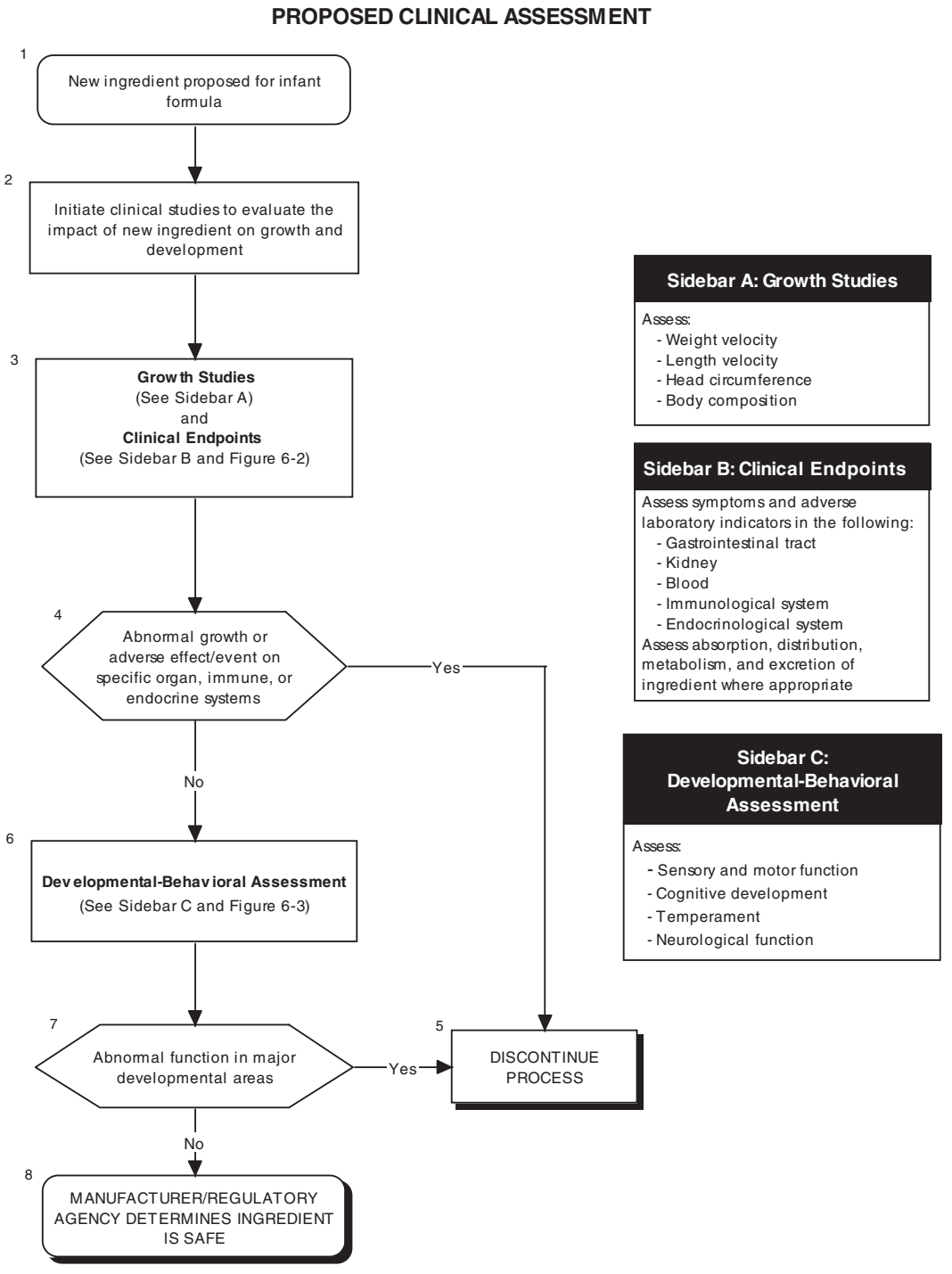

FIGURE 6-1 Proposed clinical assessment algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

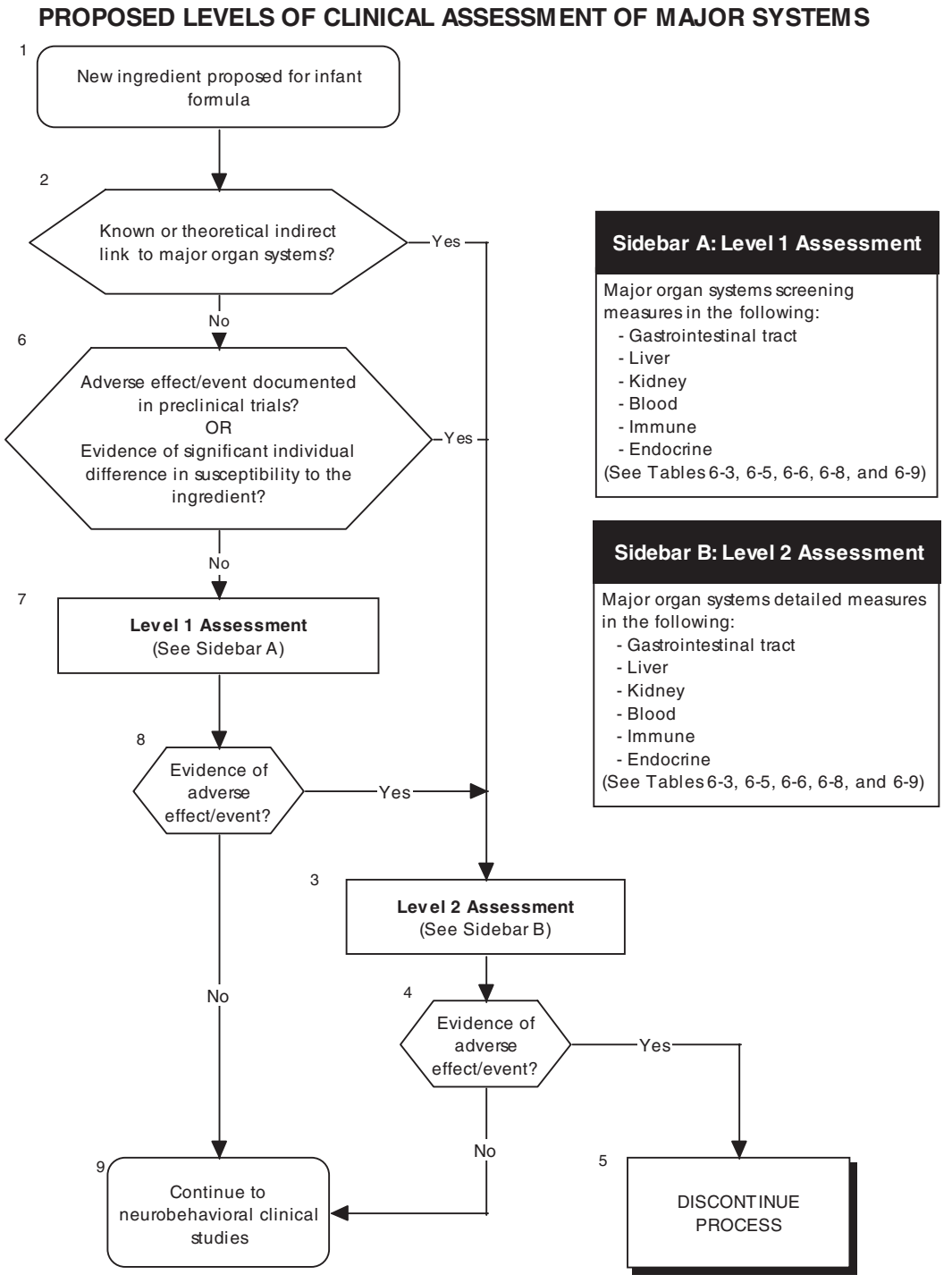

FIGURE 6-2 Proposed levels of clinical assessment of major organ, immune, and endocrine systems algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

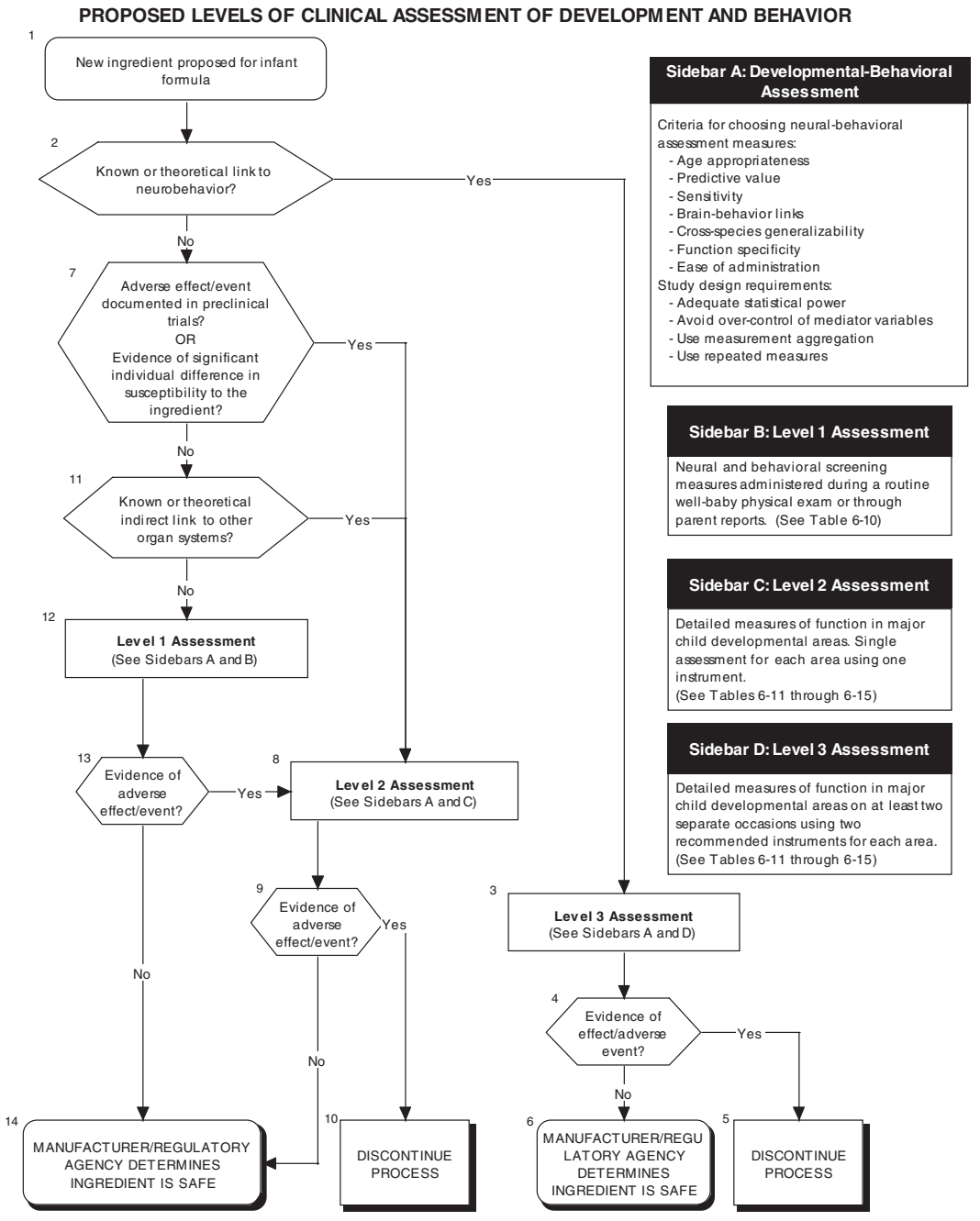

FIGURE 6-3 Proposed levels of clinical assessment of development and behavior algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

behavior. There are decision-making points within each of these three types of clinical studies that will be discussed in detail in subsequent sections of this chapter. (In keeping with the charge to the committee, proposed guidelines focus on the health and well-being of term infants only.)

The committee recognizes that all clinical studies would need to be reviewed and approved by human-subject research review boards. Because the clinical studies to determine the safety of new ingredients will be carried out in healthy infants, the committee does not recommend the use of highly invasive tests, such as tissue biopsies or gastrointestinal incubations.

OVERVIEW OF RECOMMENDED LEVELS OF ASSESSMENT

RECOMMENDATION: Any adverse systematic differences in clinical outcomes that can be attributed to an ingredient new to infant formulas should be considered a safety concern that requires careful evaluation and, if needed, further clinical study to identify the pathway through which the infant has been affected.

A hierarchy of two levels of clinical assessment should be implemented for organ systems:

- **Level 1 assessments**. Check of signs for all adverse laboratory indicators.
- **Level 2 assessments**. In-depth measures of organ systems or functions that would be performed to explain abnormalities found in level 1 assessments or specific theoretical concerns not typically addressed by level 1 tests.

A hierarchy of three levels of clinical assessment should be implemented for developmental-behavioral measures:

- **Level 1 assessments**. Developmental screening measures.
- **Level 2 assessments**. In-depth measures of infant functions in major developmental areas (single assessment for each area with one instrument).
- **Level 3 assessments**. In-depth measures of infant functions in major developmental areas (repeated assessment with multiple instruments).

GROWTH

Growth is well recognized as a sensitive, but nonspecific, indicator of the overall health and nutritional status of an infant. Monitoring infant growth has always been an integral part of pediatric care and is particularly important for young infants. Growth and nutrient requirements per kilogram of body weight are higher during the first few months of infancy than during any other period of life. Furthermore, the greatest percentage of dietary intake is devoted to supporting growth at this time, and thus nutritional imbalances are likely to be reflected in growth rates.

The committee believes that the inability of a formula to support normal growth represents a significant harm to infants and therefore growth is an essential endpoint for all safety assessments of an ingredient new to infant formulas. Any systematic deviation from normal physical growth attributable to a new ingredient should be considered a safety threat.

Under current regulations the core of the requirements focuses on meeting certain levels of specific nutrients. The concept of quality factors has not been defined, but proposed

regulations include a subsection on quality factors, with a focus on physical growth. Despite the absence of quality factors in current legislation, there appears to be a strong consensus that growth should be a quality factor for infant formulas. In the United States FDA recognized the need for clear guidelines on the assessment of growth and commissioned a report from the American Academy of Pediatrics' (AAP) Committee on Nutrition Task Force on clinical testing of infant formulas with respect to nutritional suitability for term infants (AAP, 1988). The task force identified the following types of clinical studies as useful in the premarket evaluation of formulas: acceptance or tolerance studies, gains in weight and length, food intake, body composition, serum chemical indices, and metabolic balance studies. Most of the recommendations of the task force were incorporated into the proposed changes to the infant formula act (FDA, 1996).

Currently clinical studies tend to follow the proposed rule, the 120-day growth study being the main method used to assess the ability of an infant formula to sustain normal infant growth. The proposed rule would codify standards for clinical growth studies by specifying methods (controlled clinical trials), duration (4 months), measurements (weight, recumbent length, and head circumference), and ages at measurement (at 2 and 4 weeks, then at least monthly thereafter), with a further requirement that individual infant data be plotted against Centers for Disease Control and Prevention (CDC) reference curves for weight and length.[1]

The AAP task force concluded that "rate of gain in weight gain is the single most valuable component of the clinical evaluation of infant formula" (AAP, 1988, P. 7). Further, it judged that length assessment is unnecessary because significant differences in length gain would not occur in the absence of differences in weight gain, and that there is a higher potential for measurement error and thus misclassification of growth in length. While the committee concurs with the centrality of weight gain in clinical assessment, it also believes that length and head circumference should be measured in growth studies in order to evaluate the effects of substances on other aspects of growth, such as skeletal growth and body proportions.

Notably absent from existing and proposed requirements are specific guidelines on what constitutes "normal" growth, or what represents a biologically meaningful difference among groups of infants consuming different formulas. Recommendations are needed both to define the most relevant comparison groups for clinical studies and to establish a level of difference that represents a safety concern. These are challenging and critical questions that will be discussed in later sections.

In addition, the committee recommends that guidelines go beyond growth studies to assess the safety of ingredients new to infant formulas. Deficits in brain function and effects of specific micronutrients may occur in the absence of differences in physical growth. Furthermore, while a "decrease in the growth rate during infancy is the earliest indication of nutritional failure" (Fomon, 1993, P. 48), growth deficits are likely to appear only secondary to effects on specific organs or tissues, and they may not appear for some time after nutritional insult. Thus growth studies should be considered a necessary, but not sufficient, part of human clinical studies of the safety of ingredients new to infant formulas (see Figure 6-1, Box 3).

[1]Proposed changes to 21 C.F.R. Parts 106 and 107 specify the reference charts to be used. Since CDC has published updated references for use in the United States (Kuczmarski et al., 2000), the requirement should be updated to specify the new reference values.

Measuring Growth

Ascertainment of growth status typically relies on anthropometric assessment, which is noninvasive and highly practical, requires relatively little training to achieve reliability, and is accomplished with low-cost, low-tech tools. Further, there are ample descriptions of standard anthropometric methods and reference data for the interpretation of measurements (Kuczmarski et al., 2000; Lohman et al., 1988). Although each has limitations and advantages (Table 6-1), the committee recommends the following measures of infant growth for clinical studies (see Figure 6-1, Box 3):

- *Weight* is an overall measure of body size and is responsive to acute insults, such as infectious morbidity or changes in nutrient intakes. Attained weight is hard to interpret in the absence of length data since an underweight child could be well proportioned or thin, with different implications for morbidity risk.
- *Recumbent length* is an overall indicator of linear or bone growth. Length reflects genetic factors and growth history. It is less responsive to acute insults, and the response of length to varying nutrition levels typically lags behind the response in weight.
- *Weight for length* is an indicator of relative weight (thinness or overweight). These measures are typically expressed as a *Z*-score or a percentile based on comparison with national reference data.
- *Head circumference* is often used in clinical settings as an overall, nonspecific indicator of brain growth. It has limited usefulness in screening for potential developmental or neurological disabilities, but it is useful in comparison with other anthropometrics to assess proportionality. The ratio of mid-arm to head circumference is a less commonly used index of proportionality.
- *Body composition* is a more sensitive indicator of infant nutritional status than measures of size. Depending on the method used, measurements can provide the mass of lean tissue, fat tissue, total body water, and bone. Methods vary greatly in terms of invasiveness, feasibility, cost, technology, need for trained personnel, accuracy, reliability, and precision. The most feasible methods for assessing infant body composition include anthropometry (e.g., skinfold measurements), dual X-ray absorptiometry (DEXA), and isotope dilution. A recent review concluded that for intergroup comparisons, skinfold thicknesses were useful, but for individual infant assessments, DEXA was recommended (Koo, 2000). In the absence of reference data based on a large sample of infants, the interpretation of body

TABLE 6-1 Limitations and Advantages of Recommended Growth Assessments

Recommended Assessment	Limitations	Advantages
Rate of weight gain	Nonspecific	Good global measure of infant growth and health, easy to measure reliably
Rate of length gain	Difficult to measure accurately, deficits less likely unless weight is also compromised	Provides important additional information about linear/skeletal growth and proportionality
Head circumference	Nonspecific	Easy to measure accurately, adequate global measure of head and brain growth and proportionality
Body composition	Difficult to measure accurately, best method requires expensive equipment (dual-energy X-ray absorptiometry)	More precise information about possible metabolic effects of ingredients, possible better long-term predictor of health outcomes

TABLE 6-2 Limitations and Advantages of Common Measurements of Body Composition

Method	Relevant Papers/Measurement	Limitations	Advantages
Skinfold	Schmelzle and Fusch (2002); body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry	Can be inaccurate	Rapid, low cost
Dual-energy X-ray absorptiometry	Butte et al. (1999); fat mass in infants and toddlers: comparability of total body water, total body potassium, total body electrical conductivity, and dual-energy X-ray absorptiometry	Requires expensive equipment	Rapid, precisely estimates bone mineral content, fat mass, and lean body mass
Isotope dilution		Expensive and needs specialized equipment	Noninvasive, safe

composition outcomes should rest on the comparison of groups in randomized controlled trials. Additional information on the methods used to assess body composition is provided in Table 6-2.

RECOMMENDATION: Growth studies should include precise and reliable measurements of weight and length velocity and head circumference. Duration of measurements should cover at least the period when infant formula remains the sole source of nutrients in the infant diet. Appropriate measures of body composition also require assessment.

Defining Normal Growth

The purpose of growth assessment is to determine whether a child is growing "normally." The definition of normal, inadequate, or excess growth rests largely on comparison of individual measurements with reference data that represent the distribution of sizes found in healthy infants of a given age and sex. While there is no clear cut point to define a size at which there is an abrupt elevation in risk of poor outcomes, measurements that fall above the 95th or below the 5th percentiles of an accepted reference are typically cause for concern. While short periods of abnormal growth rate may not be of concern, low or high rates over several months may be related to increased morbidity risk, both in the long and short term. Therefore a single measurement of attained size at a given age is not a sufficient measure of growth. Repeated, appropriately spaced measurements are needed to calculate growth rate. A clinical assessment of infant growth for the purpose of determining the safety of an ingredient new to infant formulas must therefore be based on a longitudinal study, with repeated measures at relatively frequent intervals during the period when growth is most rapid and during the time period when formula serves as the sole source of infant nutrition.

Identifying Appropriate Comparison Groups

As discussed in Chapter 3, there are challenges in selecting appropriate comparison groups for clinical studies to assess the safety of infant formulas. The gold standard design—the double-blind, randomized, controlled trial—randomly assigns comparable groups of

infants to receive either the formula containing the new ingredient or a previously approved formula. Implicit in this design is the assumption that infants fed the approved formula form the appropriate comparison group. However when testing for deviation from *optimal* infant growth, the appropriate comparison group should be one that demonstrates optimal growth. Since growth of healthy breastfed infants is considered optimal, then exclusively breastfed infants form the most appropriate comparison group. The committee recommends using dual control groups—breastfed infants and infants fed the previously approved formula without the new ingredient—in order to ensure a thorough analysis.

Breast versus formula feeding cannot ethically be randomly assigned, nor could these feeding conditions be blinded. Instead, reference data from healthy breastfed infants, measured at comparable intervals using identical methods, can be used for comparative purposes. This would allow multiple intergroup comparisons that would put differences between two infant formulas in perspective relative to formula-breastfeeding differences, as has been done in trials of formula containing long-chain polyunsaturated fatty acids (LC-PUFAs) (Auestad et al., 2001). The World Health Organization is currently working to create a growth reference for breastfed infants that should be suitable for such comparisons in the future (Garza and de Onis, 1999).

Estimating Intake

All clinical trials must include an estimation of daily formula intake in order to determine which effects are the result of different levels of intake and which are to the result of the specific ingredient. For example, if an ingredient alters taste or palatability, it may change the level of intake.

Specifying a Level of Difference in Growth That Represents a Safety Concern

This is important for interpreting average group differences, as well as deviations from normal growth in an infant that are attributable to being fed a different formula. There is very little scientific evidence to establish a level of difference associated with long- or short-term health consequences. The AAP task force (AAP, 1988) recommended that a weight gain difference greater than 3 g/day over 3 to 4 months should be considered nutritionally significant. Over 3.5 months, this would represent a difference of about 320 g. This is less than the difference between the 25th and 50th percentile and equivalent to the difference between the 90th and 95th percentile of weight at age 3.5 months for boys based on the CDC growth charts (Kuczmarski et al., 2002). No specific evidence to support this level of difference was provided. In a clinical setting, a diagnosis of failure to thrive is based on rate of weight gain and weight-for-length status interpreted in the context of growth history. Fomon (1993) defines failure to thrive in comparison with U.S. reference data for weight increments and weight-for-length status. He uses two standard deviations relative to 2-month increment data for infants under 6 months of age and a weight-for-length below the 5th percentile as cutpoints.

For perspective, it may be useful to compare reported differences in growth rates of healthy breastfed and formula-fed infants. Data from Fomon's infant growth studies (Nelson et al., 1989) show differences of 2.4 g/day in boys and 1.3 g/day in girls who were breastfed versus formula fed from 8 to 112 days of life. This would result in a 250-g difference in boys and a 135-g difference in girls over the 104 days. Based on more recent data, Dewey and colleagues (1992) compared growth of infants in the DARLING study and found consistently higher weight velocities (g/mo) in formula-fed versus breastfed boys in the first 6

months of life, but no significant effects of feeding mode on weight velocity in girls. The cumulative effect of the differences in weight velocity among boys amounted to about 284 g over a period of 4 months. Furthermore, infants who were breastfed for 12 months or more were leaner, had smaller skinfolds, and had a lower percent body fat. These differences persisted into the second year of life (Dewey et al., 1993).

Kramer and colleagues (2002) recently reported results of a large study of Belarus infants and found that infants who were exclusively breastfed for at least 3 months had weight and length *Z* scores that were about 0.2 standard deviations above those of infants who were weaned in the first month of life. Using data from the Third National Health and Nutrition Examination Survey, Hediger and colleagues (2000) found no differences in weight status by feeding method in 4- to 7-month-old children, but between 8 and 11 months of age, infants who had been exclusively breastfed had weights that were about one-fifth of a standard deviation below the U.S. reference median. This would represent a 200-g difference in growth associated with breastfeeding among infants of average size.

In comparison with published growth velocity reference data from the Fels Longitudinal Study (Guo et al., 1991), 3 g/day roughly represents the differences between the major percentile lines in the 3-month increment data (e.g., for boys, the 25th, 50th, and 75th percentiles were 23, 27, and 31 g/day, respectively). The clinical or functional significance of such differences is not well established.

Body composition is not typically assessed as a part of normal well-child care in clinical settings. The interpretation of body composition measures has been particularly challenging because extensive reference data on infants is lacking, and few studies have attempted to identify specific health risks associated with levels of body fat or lean tissue. More than a decade ago, the AAP task force concluded that methods to determine body water, body fat, and bone mass had "not reached the stage of precision, noninvasiveness and convenience that would make them feasible as a part of routine clinical testing of infant formulas" (AAP, 1988). However the state of the art has changed dramatically since then, and it is now possible to assess body composition using a variety of minimally invasive and precise methods. Furthermore, Butte and colleagues (2000) recently published reference data for infant body composition using a four-compartment model to estimate fat and fat-free mass, a deuterium dilution to measure total body water, and DEXA to measure bone mineral content.

It is important to evaluate body composition in the context of safety. Ultimately the goal of assessment is to identify levels of difference in body composition that are associated with immediate or long-term disease risk. The relevant component of body composition to measure will depend on the nature of the added ingredient. For example, if an ingredient is likely to have metabolic effects, it will be important to assess the relative contribution of fat and fat-free mass since these components may differentially reflect underlying factors related to energy and protein balance. In contrast, for other ingredients, bone mineral content may be more relevant.

Interpreting Inadequacies and Excesses in Growth Outcomes

The health implications of inadequate growth increments are not well described except in the context of severe undernutrition, which is an event unlikely to occur in closely monitored infant-feeding trials. There is evidence, primarily from populations with high poverty levels, of an association between more severe length and weight deficits (stunting and wasting) and impaired immune function (Forse et al., 1994), increased risk of morbidity and mortality (Pelletier et al., 1993), and poor developmental outcomes (for review, see

Grantham-McGregor et al., 2000). Mild growth deficits tend not to be strongly related to specific health outcomes independent of other nutritional risk factors. In comparisons of breastfed and formula-fed infants, despite differences in growth patterns, Dewey and colleagues (1991) found no differences in behavior or activity levels of breastfed and formula-fed infants.

While the focus in the past has been on nutritional inadequacies or growth deficits associated with formula feeding, it is important to also assess the potential for a new ingredient to cause excess growth. Aside from the risks associated with macrosomia in newborns, there is a lack of information on the immediate consequences of excess weight or of differences in body composition during infancy. While not related to feeding, infants with macrosomia associated with maternal gestational diabetes are at increased risk of postnatal obesity. This would seem to be an effect of the mother's diabetes on body composition, with macrosomic infants having a significantly higher percent body fat (Fee and Weil, 1960). Of greater concern is the long-term consequences of excess infant growth, particularly in light of the worldwide epidemic of child and adult obesity. There is inconsistent evidence that fatness or excess weight gain in infancy predicts later obesity. When associations between excess infant growth and later outcomes do exist, they could reflect genetic factors or common behaviors, such as a consistent tendency of parents to overfeed.

The best evidence supporting an association between rapid infant weight gain and later risk of overweight comes from a prospective cohort study of more than 19,000 participants in the National Perinatal Collaborative Study. Researchers assessed the relationship of weight gain in the first 4 months of life to overweight at age 7 years (defined as body mass index [BMI] > 95th percentile of the CDC growth charts). After adjusting for birthweight, gestational age, sex, race, firstborn status, maternal BMI, and maternal education, they found that for each 100-g weight gain increase per month, the risk for overweight at age 7 increased by about 30 percent (Stettler et al., 2002b). Furthermore, nearly one-fifth of overweight status at age 7 could be attributed to infancy weight gain above the highest quintile. Stettler and colleagues (2002a) also found that weight gain in the first year of life was strongly associated with overweight and obesity in the school years among children living in the Seychelles. In a large British cohort born in the 1990s, more rapid weight gain in the first 2 years of life, evidenced by an increase in a weight-for-age *Z* score of greater than 0.67, was associated with higher BMI, percent body fat, and total fat mass in later childhood (Ong et al., 2000). In Pima Indian children, a population with a very high prevalence of obesity and type 2 diabetes, Lindsay and colleagues (2002) found two periods characterized by excess weight gain relative to the CDC growth reference. These were from 1 to 6 months and 2 to 11 years of age. Although Dietz (1994) did not identify infancy as one of the three critical periods in childhood for the development of obesity, the more recent findings summarized above have led researchers to suggest that infancy represents another critical period for the development of obesity later in life.

In contrast, there are a number of studies that find no evidence that overweight babies are destined to become overweight adults, unless they have obese parents. For example, Whitaker and colleagues (1997) found that risk of obesity in young adults was not increased by obesity at age 1 to 2 years unless at least one parent was also obese. Infancy is characterized by a substantial capacity for compensatory growth following a period of failure to achieve growth potential or a period of excess growth, thus limiting the long-term consequences of relatively short periods of abnormal growth. Butte and colleagues (2000) compared multiple dimensions of body composition among breastfed and formula-fed infants. Despite significant differences in early infancy, they found no persistent difference by feeding method beyond age 12 months.

There remains controversy over the extent to which deficits or excesses in overall growth, growth of specific organs and tissues, or differences in fat versus lean tissue have long-term effects on physiological functioning and disease risk. Again, the evidence of long-term effects tends to focus on the extremes in child size. For example, stunting in childhood is associated with short stature in adults, which is in turn associated with lower work capacity among adults engaged in physically demanding jobs and increased risk of poor obstetric outcomes in women. However a recent study of a cohort of Finnish children was the first to show that infant obesity was significantly associated with later development of type 1 diabetes (Hypponen et al., 2000). The hypothesized mechanism is hyperinsulinemia and damage to beta cells associated with early excess body fat. Based on research among Indian infants, Yajnik (2001) has hypothesized that deficits in skeletal muscle in infancy may contribute to insulin insensitivity and risk of type 2 diabetes later in life.

There is increasing evidence that growth deficits in utero and in the early postnatal period have important long-term health consequences owing to "programming" of structure or metabolic functioning by nutritional inadequacies. The focus of most of the research has been on fetal programming (Godfrey and Barker, 2001), but there is also evidence of effects of postnatal growth deficits resulting in small size at 1 year of age (Vijayakumar et al., 1995). Furthermore, there is evidence to suggest that feeding mode during infancy has long-term effects on lipid profiles (Cowin and Emmett, 2000; Plancoulaine et al., 2000), risk of later obesity (Armstrong and Reilly, 2002; for a review of effects on obesity, see Butte, 2001), and risk of other diseases (Leeson et al., 2001). However there is no particular substance in milk to which these effects may be attributed.

Evaluating the 120-Day Growth Study

Although not currently a requirement, manufacturers often provide 120-day growth studies to demonstrate healthy growth.[2] The committee was specifically asked to evaluate the adequacy of the currently used 120-day growth study. Conceptual issues on the measurement of growth are discussed above, so the following section is confined to a discussion of the duration of the study. The first 120 days of life is a period during which infant formula is most likely to be the sole source of nutrition for the infant and a period of high growth rates and, thus, a period of high susceptibility to dietary intake. From a practical perspective, it may be difficult to recruit infants whose parents are willing to forego the introduction of other foods until after 6 months of age, and there is no reason to think that an adverse effect of an ingredient new to formulas would be detected *only* between 4 and 6 months of age. However a study length of 120 days may be insufficient for several reasons. First, human milk is recommended as the sole nutrient source for infants for at least the first 4 months (AAP, 1997; IOM, 1991) and preferably for the first 6 months of life (ADA, 2001; IOM, 1991; WHO, 2002). When intended as a human-milk substitute, exclusive formula feeding should be recommended for the same period of time. Ideally formula should be tested for the entire period for which it is intended to be fed as the sole source of infant nutrition, consistent with breastfeeding guidelines. This is particularly true since intake of infants receiving only formula will be greater in the period from 4 to 6 months of age.

[2]FDA published a proposed rule that would change several aspects of the infant formula regulations. FDA is proposing to revise "Quality Factors for Infant Formulas" (FDA, 1996). Among other requirements, FDA is proposing to establish healthy growth as a quality factor. To ensure that the new infant formula supports normal physical growth, the proposed rule would require that growth studies be performed for 120 days.

Second, serious limitations of the 120-day growth study are that it does not allow for the determination of delayed effects or for understanding longer-term effects of early perturbations in growth. Longer-term follow-up of participants in clinical studies should be recommended, with the duration to cover at least the period when infant formulas remain a substantial source of nutrients in the infant diet.

In summary, the committee recognizes that to establish levels of growth that indicate a safety concern is a difficult endeavor. However the committee concludes that any systematic and statistically significant difference in size or growth rate between infants fed a formula containing a new ingredient versus human milk or a previously approved formula should be a safety concern.

SPECIFIC ORGAN SYSTEMS

The Importance of Assessing Specific Organ Systems

As described in the previous section, the committee recommends that growth studies should remain the centerpiece of clinical testing of ingredients new to infant formulas. However growth deficits are likely to appear only secondary to effects on specific organs or tissues and may not appear for some time after nutritional insult. The major organ systems should also be studied when assessing the safety of an ingredient new to infant formulas (see Figure 6-1, Box 3 and Figure 6-2).

The gastrointestinal tract is the first organ that encounters ingested ingredients. It serves to protect the infant from environmental pathogens, antigens, toxins, and other noxious agents (Walker, 2002). In healthy infants, gastrointestinal tract functions assure that normal growth and development occur, provided the infant is offered the necessary nutrients. Any of these functions may be affected by the diet or ingredients in the diet, and impaired function can lead to inadequate nutrient availability.

The immune system is also important during infancy because of its regulatory effects of a substance on immune competence and the potential for an inflammatory or allergic response to a new ingredient. Food allergy and other adverse reactions to food are more common in infants than in any other age group. This is partly a reflection of the relative immaturity of the infant immune system. Compared with older children and adults, young infants have low immunoglobulin A concentrations and thus reduced binding of antigens in the gut. The infant immune system is not fully mature at birth, with deficits in the ability to prevent invasion of pathogens and to respond to antigens. Of particular concern in the context of ingredients new to infant formulas is the increased permeability of the gut mucosal barrier in the presence of inflammation or infection or if the integrity of the epithelial cell layer is disrupted. The increased permeability allows macromolecules to be absorbed and stimulates allergic responses to food proteins. Furthermore, immature lysosomal function in mucosal cells and limited intracellular proteolysis may result in further intestinal damage and increased permeability.

Finally, the endocrine system is not fully developed until after puberty has occurred. It is possible that an ingredient new to infant formulas could affect endocrine development or expression of endocrine function. An example of such a possibility is the phytoestrogen content of an infant formula having an effect on the development or expression of estrogen-responsive tissues. Therefore clinical studies should include additional assessments to ensure that infants:

- grow and develop according to standards,

- consistently display normal vital signs,
- feed and stool normally,
- do not vomit (aside from infantile reflux),
- demonstrate consistently normal laboratory values (blood count, blood chemistries, liver function, renal function, and urine analysis),
- do not present immunologically related injuries, and
- do not present signs of endocrine disruption.

RECOMMENDATION: Assessment of clinical endpoints should include signs or adverse laboratory indicators of the major organ systems, including the gastrointestinal tract, kidneys, blood, and immunological and endocrinological systems.

Assessing the Gastrointestinal Tract

The gastrointestinal tract consists of the hollow organs (mouth, pharynx, esophagus, stomach, small bowel, and colon) and the solid organs (liver and pancreas). The functions of the hollow organs are as follows:

- Motility. The propulsion of lumenal contents through the gastrointestinal tract occurs as the result of contractions of two layers of perpendicularly oriented smooth muscle. Beginning in the esophagus, the movement of the walls of the hollow tube mixes and propels lumenal contents. Nutrients and water are absorbed and waste is extruded during the passage of substances through the tubular gastrointestinal tract.
- Digestion and absorption. Digestion begins in the mouth with salivary enzymes and continues through the colon, where some digestion of carbohydrate can occur, especially in infants. A relative fat malabsorption occurs in infants compared with adults (Fomon et al., 1970). Similarly, pancreatic secretion of amylase and starch digestion is less in infants than in adults. Absorption of nutrients occurs throughout the gastrointestinal tract, beginning in the small bowel. Absorption can be passive (diffusion), active, or carrier mediated.
- Secretion. Secretion of substances, such as acid, pepsin, bile, and enzymes, is necessary to digest nutrients. In addition to the digestive material, the gastrointestinal tract secretes hormones and paracrine substances that modulate the function of other cells.

With the exception of pancreatic exocrine function and bile acid synthesis and composition, development of the gastrointestinal tract is essentially complete at birth for infants born after a 34-week gestation (Antonowicz and Lebenthal, 1977; Auricchio et al., 1965; Fredrikzon et al., 1978; Hadorn et al., 1968; Hamilton, 2000; Lindberg, 1966; Montgomery et al., 1999; Norman et al., 1972; Watkins, 1985; Watkins et al., 1973).

Clinically relevant tools are available to assess each function of the gastrointestinal tract and some tools are more specific than others. For example, normal growth and development occurs only when the gastrointestinal tract is functioning optimally. But slowed or inadequate growth, as the common denominator of impaired gastrointestinal function, does not identify the function that is impaired. Table 6-3 lists the functions of the gastrointestinal tract and general (level 1 assessments) and specific (level 2 assessments) clinical outcome measures that can be used to assess whether a specific function has been impaired. Table 6-4 lists the advantages and disadvantages of each of the outcome measures.

Motility can be assessed by measurement of esophageal, antroduodenal, small bowel, and rectal contractions (Scott, 2000); gastric emptying time (Di Lorenzo et al., 1987); and

TABLE 6-3 Gastrointestinal Tract Clinical Endpoints

Function	Level 1 Assessments	Level 2 Assessments[a]
Absorption	Growth velocity	Stool fat, stool protein, stool carbohydrate, stool alpha-1-antitrypsin, stool pH, balance studies, blood levels of specific nutrients
Allergic	Vomiting, diarrhea, irritability, colitis	Serum IgE, quantitate peripheral eosinophils, skin tests, RAST, challenge tests
Barrier	Not applicable	Polyethylene glycol, lactulose/mannitol, ^{51}Cr EDTA, proteins, stool cultures, serum antigens
Biotransformation	Serum liver enzyme levels	Blood levels of bile acids or specific drugs and their metabolites, serum liver enzyme levels, isotope excretion scans
Digestion	Growth velocity	Stool: fat, protein, sugars, pH
Homeostasis	Not applicable	Balance studies, blood/tissue levels of specific nutrients
Immunological	Serum antibodies	Response to oral vaccinations, stool cultures
Motility	Absence of vomiting, stool pattern, growth velocity	Measurements of transit times (e.g., charcoal, hydrogen breath tests)
Secretory	Growth velocity	Measure specific hormones, stool chymotrypsin, elastase, alpha-1-antitrypsin
Metabolism of macronutrients		
Protein	Growth velocity, serum liver enzyme levels, liver size by examination	Urine and serum amino acid levels, serum glucose serum proteins (e.g., albumin, prealbumin, clotting factors, alpha-1-antitrypsin, transferrin), serum liver enzyme levels, liver size by examination and ultrasound
Lipid	Liver size by examination, serum liver enzyme levels	Serum lipid levels (cholesterol, phospholipids, triglycerides), serum glucose, liver size, and fat content by examination and ultrasound, urine organic acids
Carbohydrate	Liver size by examination, serum liver enzyme levels	Serum glucose levels, serum liver enzymes, liver size by examination and ultrasound

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]pH = potential of hydrogen, IgE = immunoglobulin E, RAST = radioallergosorbent test, EDTA = ethylene-diaminetetraacetic acid.

transit time (Scott, 2000). The muscle fibers, nervous tissue, and some neurotransmitters can be evaluated with biopsies. Generally, however, motility is functionally normal if there is no vomiting, if the stool pattern is normal, and if growth velocity is normal. If measurements of motility are needed they should be carried out by noninvasive techniques.

Digestion and absorption can be monitored by quantifying stool fat (Fomon et al., 1970; van de Kamer et al., 1949); protein, such as alpha-1-antitrypsin (Dinari et al., 1984); carbohydrate content (Grant et al., 1989); and breath tests (Fernandes et al., 1978; Maffei et al., 1977; Perman et al., 1978; Robb and Davidson, 1981; Thomas et al., 1981). The amount of specific nutrients can be quantified in blood. However if growth velocity remains normal, it is unlikely that digestion is adversely affected by dietary intake.

Some secretory functions of the gastrointestinal tract can be assessed by quantifying levels of specific hormones or enzymes (e.g., gastrin, cholecystokinin, trypsin, lipase, or motolin) in the blood or stool. Growth velocity falters if the secretory functions are impaired.

For some nutrients the gastrointestinal tract regulates absorption based on nutrient

TABLE 6-4 Advantages and Disadvantages of Various Organ Clinical Endpoints

Outcome Measure	Advantages	Disadvantages
Balance studies	Accurate assessment of specific nutrients	Requires admission to a clinical research center
Bleeding time	Easy, safe, accurate	Painful, requires a small incision, does not identify specific clotting abnormality
Blood pressure	Noninvasive, used in routine health assessment, standards available for children	Requires personnel with some training
Dual-energy X-ray absorptometry scan (bone mineralization)	Accurate for bone mineralization, fat mass, and lean body mass	Normative data not available for infants, may require sedation, requires expensive equipment
Fecal fat–72 hour	Noninvasive	Collection starts and ends with marker, in-home collection may be difficult, collection in a clinical research center is more accurate
Growth velocity	Established normal values, noninvasive, inexpensive, readily available technology	Does not identify specific function that is deficient or impaired
Motility	Specific, can identify area of the gastrointestinal tract where an abnormality occurs	Invasive, limited to centers, time intensive in infants who cannot cooperate, but can measure some aspects by noninvasive techniques (e.g., hydrogen breath tests)
Permeability studies (polyethylene glycol, sugars, antigens)	Easy to use probes	Requires urine or blood collection
Serum levels of nutrients, hepatic enzymes, bile acids, chemistries, blood gas, blood counts, specific proteins	Accurate measure of circulating nutrient levels, liver function, hematological function	Requires blood draw, some assays available only in centers
Stool components (fat, enzymes, protein, pH, reducing substances, cultures)	Noninvasive, relatively easy to collect in infancy	Depending on the test, varying specificity and sensitivity
Urine analysis, quantitation of nutrients, estimate of glomular filtration	Noninvasive, accurate	Collection may be difficult in infants
Ultrasound	Noninvasive, gold standard to size abdominal organs and assess cirrhosis, fatty infiltration in liver, and inflammation in bowel	Relatively expensive
Vaccine response	Accurate measure of B-cell function	Requires injection of vaccine, blood draw

levels. This regulation is often complex and involves other organs, such as the liver and kidney for calcium homeostasis (IOM, 1997), and the liver, spleen, and bone marrow for iron (IOM, 2001). Balance studies, stable isotopes, levels of specific nutrients in blood or tissue, and storage forms of specific nutrients can be assessed.

The gastrointestinal tract is the site at which interaction with a food allergen occurs.

Different factors predispose for the development of food allergy, such as family history, immune deficiency, or early exposure to antigens. Food allergy can consist of type I, III, or IV reactions. Allergic reactions of this organ include enteropathy, colitis, and nonspecific reactions, such as recurrent vomiting, bowel edema, obstruction, constipation, occult bleeding, and colic. The manifestations of food allergy vary with age and site of food antigen exposure. In infancy food allergy can be assessed by evaluating the infant for vomiting, diarrhea, malabsorption, gastrointestinal loss of blood or protein, and constipation, and by performing challenge tests.

It is unlikely that the human term-infant gastrointestinal tract is more permeable than that of older infants and children (Sanderson and Walker, 1993). One study using human α-lactalbumin as a marker of permeability showed that serum concentration of this protein was increased in term breastfed infants for the first several months of life (Jakobsson et al., 1986). However others, using bovine β-lactoglobulin in formula-fed infants, did not show a change in gastrointestinal permeability over the first several months of life (Roberton et al., 1982). In healthy term infants, gastrointestinal permeability may be increased by allergy (Boehm et al., 1992; Dupont et al., 1989; Falth-Magnusson et al., 1986; Heyman et al., 1988; Juvonen et al., 1990; Schrander et al., 1990), infection (Holm et al., 1992), and perhaps colic (Lothe et al., 1990). Permeability can be assessed by using the inert carbohydrates (e.g., lactulose and mannitol), polyethelene glycol 4000, ^{51}Cr ethylenediaminetetraacetate, and heterologous proteins (e.g., bovine β-lactoglobulin) or homologous proteins (e.g., human α-lactalbumin) (Bjarnason et al., 1995; Sanderson and Walker, 1993).

The pancreas serves as a secretory- (exocrine) and hormone- (endocrine) producing organ. Exocrine functions are difficult to assess in clinical studies in healthy infants but, as noted above, can be assessed by directly quantifying lumenal concentrations of enzymes and bicarbonate before and after a stimulus. It is only when pancreatic exocrine secretion is dramatically decreased that a deceleration of growth velocity occurs (Huynh and Couper, 2000). Severe pancreatic insufficiency can be monitored by measuring fat or certain enzymes (e.g., trypsin) in stools. Pancreatic endocrine dysfunction is most often manifested as diabetes, which can be assessed by obtaining serum insulin concentrations, blood, and urine glucose (Huynh and Couper, 2000).

The liver plays a central role in the metabolic adaptation of the fetus to extrauterine life through glucogenolysis, gluconeogenesis, and the regulation of amino acid and fat metabolism (Karpen and Suchy, 2001). These functions of the liver can be assessed by quantifying urine and blood amino acid levels; urine organic acid levels; blood proteins, lipids, ammonia, and bicarbonate; liver fat; and ultrasound. However if the liver is unable to function normally with respect to carbohydrate, protein, or lipid metabolism, normal growth velocity will not be maintained.

In addition, the liver synthesizes and excretes bile acids (Setchell and O'Connell, 2001). Bile acid synthesis, the bile acid pool, and intralumenal bile acid concentrations gradually increase during the first year of life. Bile acid secretion is maximal at birth and cannot be further stimulated. This function of the liver can be assessed by quantifying blood levels of liver-derived enzymes as a marker of hepatocyte integrity, by quantifying blood levels of bile acids and isotope excretion scans as a marker of hepatic excretory function, and by measuring serum levels of specific drugs and their metabolites (Batres and Maller, 2001).

Assessing the Kidneys

The kidneys perform vital functions, including filtration of plasma, reabsorption of water and electrolytes, excretion of wastes, and the production of hormones that control

TABLE 6-5 Kidney Clinical Endpoints

Function	Level 1 Assessments	Level 2 Assessments
Filtration	Growth velocity, urine analysis, serum creatinine	Glomular filtration rate
Reabsorption	Growth velocity, serum acid base, serum electrolytes, urine analysis	Plasma acid base, serum electrolytes, urine analysis
Endocrine	Blood pressure, urine analysis, bone mineralization assessment, serum calcium, phosphorus	Vitamin D, serum calcium, phosphorus, magnesium, erythropoetin, prostaglandins, renin, angiotensin, kallikrein, kinin, bone mineralization assessment

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

blood pressure, calcium homeostasis, and red cell production (Binley et al., 2002; Gleim, 2000; McMurray and Hackney, 2000). Specific tests (level 2 assessments) can be performed to identify each of these functions, but general assessments (level 1 assessments) of blood pressure, urinary analysis, growth velocity, serum creatinine, blood urea nitrogen, calcium, bicarbonate, and a complete blood count will establish if renal function is abnormal or adversely affected by a component of the diet (Table 6-5).

Specific functions of the kidney that can be assessed include glomerular filtration rate (GFR), which can be measured by quantifying the clearance of a substance that is freely filtered across the capillary wall and is neither reabsorbed nor secreted by the tubules. The optimal measurement of GFR is insulin clearance (Arant et al., 1972). Clinically, however, GFR can be estimated by the clearance of endogenous creatinine. At serum levels of creatinine exceeding 2.0 mg/dL, changes in renal function can be monitored by the serum creatinine concentration. GFR is adequate for healthy term infants, but it does not approximate adult rates until about 3 years of age. Renal tubular reabsorption and urine acidification is less at birth and for several months thereafter than it is for adults. This function is adequate for healthy infants, but contributes to fluid and electrolyte abnormalities in infants who are ill or are fed an inappropriate diet (Goldsmith and Novello, 1992).

The kidney also serves as an endocrine organ, synthesizing and degrading prostaglandins, kallikrein-kinin, and renin-angiotensin, which control blood pressure. Hydroxylation of vitamin D creates the hormone that controls calcium homeostasis, which occurs in the kidney. Erythropoetin, the glycoprotein that regulates both steady-state and accelerated red blood cell production, is governed by oxygen availability to the kidney. Blood levels of these hormones and the substances they regulate can be quantified.

Assessing the Blood

The hematological system consists of red blood cells, white blood cells, platelets, and proteins. The function of the red blood cell is to transport oxygen to tissues. This function is performed by hemoglobin, which combines reversibly with oxygen, allowing the red blood cells to transport oxygen from the lungs and deliver it to tissues. Hemoglobin accounts for more than 95 percent of the total protein and about 90 percent of the dry weight of the red blood cell. Red blood cell function can be assessed by quantifying the number of cells, the hemoglobin concentration, and the hematocrit. Membrane lipid analysis, fragility studies, observation of the blood smear, and a reticulocyte count can also be performed (Brugnara and Platt, 1998).

The capacity of white blood cells to produce antibodies to antigens is intact at birth. In general, white blood cell function can be assessed by quantifying the total white cell count, the absolute count of specific cells, skin tests, and immunoglobulin levels. The specific function of phagocytic cells, such as chemotaxis, ingestion, and oxidative metabolism, can be assessed in isolated cells. Some products of these functions, such as myeloperoxidase, can be quantified in blood or stool. Specific lymphocyte function can also be assessed in isolated cells and by quantifying inflammatory mediators in blood. Abnormalities in white blood cell function can be suspected clinically by occurrence of frequent infections or infections caused by low-virulence pathogens.

Platelets are important in homeostasis. Platelet activity is assessed by quantifying the number and morphology of platelets in a blood sample and by assessing platelet aggregation and specific platelet functions. Platelet function can also be assessed by performing a bleeding time (Handin, 1998).

Several clotting factor concentrations in blood are lower during the neonatal period than in adulthood (Esmon, 1998). The lower level of clotting factors is associated with prolonged prothrombin and partial thromblastin time. After the neonatal period, coagulation is the same as that of adults. Coagulation function can be assessed by quantifying each of the following factors: I through XII, plasminogen, antithrombin III, prekallikrein, and high molecular weight kininogen. Coagulation can also be assessed by performing a thrombin time and a partial thromboplastin time or by noting if abnormal bleeding is present (see Table 6-6).

Assessing Immunological and Allergic Activity

A newborn's digestive system is fairly mature, but may only incompletely break down food proteins. Thus infants are especially susceptible to allergic sensitization. The immune response of newborn infants is predominantly associated with the Th2-type of the helper T-cell population, possibly because of in utero priming of fetal T cells by transplacental passage of common environmental allergens and dietary antigens. In general normal infants exhibit a low-grade immunological response to subsequent exposure to such environmental

TABLE 6-6 Blood Clinical Endpoints

Function	Level 1 Assessments	Level 2 Assessments
Oxygenation	Complete blood count, clinical color (pallor, ruddiness, cyanosis)	Red blood cell number, hemoglobin/hematocrit, membrane lipids, fragility, reticulocyte count, red cell fragility
Immune defense phagocytosis	Frequency of natural infections	White blood cell number, differential count, chemotaxis, ingestion, oxidative metabolism, absolute polymorphonuclear count
Immune defense lymphocytes	Frequency of natural infections	White blood cell number, differential count, delayed sensitivity, skin tests, immunoglobulin levels, absolute lymphocyte count
Bleeding	Bleeding time	Platelet numbers, in vitro bleeding, platelet aggregation, clotting factors, thromboplastin time, partial thromboplastin time

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

agents after birth, which is limited to immunoglobulin (Ig) G and IgM isotypes and to the Th1-type of the helper T-cell population (Holt et al., 1999). During further development the neonatal immune system continues to shift towards Th1-type response. It has been proposed that alterations in the neonatal mucosal environment (e.g., a change in microflora), the use of formulas (and lack of breastfeeding), antibiotics, mucosal infections, and a highly hygienic environment in early infancy may lead to further increase in the Th2-type of helper T cells that were primed in utero (Holt et al., 1999). Th2-type helper T-cell expression is currently considered the hallmark of allergic immunopathology (Kay, 2001).

Many of the proteins added to infant formulas are functional proteins (i.e., proteins that are added for their function—not as a source of amino acids) and to maintain their function, they must be resistant to digestion, a property shared with allergens. The addition to infant formulas of novel proteins (including glycoproteins or lipoproteins), which by their nature can induce allergic or other adverse reactions, requires clinical testing. Human-milk proteins are not expected to be allergenic in humans since they are produced by the human mammary gland. Possible exceptions are the proteins that originate from maternal dietary components, such as cow-milk proteins. Also, as has been recognized in the production of biotech crops (Kok and Kuiper, 2003), the commercial production of milk proteins using recombinant technologies may produce unintended and unexpected side effects. For instance, one milk protein produced in recombinant microorganisms may differ from native proteins in level of glycosylation, posttranslational modifications, or minor amino acid sequences, which may change the allergenicity potential. Furthermore, there is potential for contamination with compounds deriving from the genetically modified organism used as the protein source.

The central aspect of clinical testing in infants should include the evaluation of a diverse spectrum of immune functions in response to an added substance. To develop the appropriate tests for assessing the safety of the immunological responses to new substances, it is useful to first identify the target tissues affected by the interaction of ingested substances with the host immune system (Table 6-7).

TABLE 6-7 Target Tissues and Signs Derived from Interactions of a New Ingredient with a Host Immune System Target Tissue

Target Tissue	Immunoglobulin E-Mediated	Mediated by Other Immunological Mechanisms
Gastrointestinal	Infantile colic	Food-induced enterocolitis and proctocolitis
	Eosinophilic gastroenteritis	Allergic gastroenteritis, eosinophilic (postprandial nausea, weight loss)
	Oral allergy (angioedema)	Celiac-like disease
	Gastrointestinal tract anaphylaxis (nausea, chronic diarrhea)	
	Celiac disease	
Airway	Rhinitis-conjunctivitis	Heiner syndrome (pulmonary hemosiderosis)
	Laryngeal edema-obstruction	
	Acute bronchospasm	
Skin, joint, blood vessels	Urticaria	Dermatitis herpetiforms
	Atopic dermatitis	Contact sensitivity
		Contact irritation (acidic fruits and vegetables)
		Migraine
		Arthritis

SOURCE: Sampson (1996, 2002).

When measuring immunocompetence, clinical assessment of allergic response should include evaluation of specific signs, laboratory testing for evidence of specific immune responses, inflammatory cytokines, and allergen-specific response to challenges. IgE isotype is present at birth and, therefore, specific signs of IgE-mediated allergic reactions, such as urticaria, vomiting, diarrhea, respiratory signs, and anaphylaxis, can occur in the neonate. Clinical signs of atopic dermatitis, including erythema, edema, crusts, excoriations, lichenification, dryness, degree of itch, and loss of sleep, should be evaluated. The latter are evaluated using a scoring system for the extent and intensity of dermatitis (European Task Force on Atopic Dermatitis, 1993). Gastrointestinal responses, particularly the chronic type, are generally presumed to be T-cell mediated, but may also be associated with specific IgE-mediated immunological interactions. These include enterocolitis, proctocolitis, enteropathy, and a subset of allergic eosinophilic esophagitis/enteropathy (e.g., vomiting and diarrhea) with eosinophil infiltration of the affected portion of the gastrointestinal tract (Sicherer et al., 2001).

Laboratory testing for evidence of specific immune responses should include the determination of serum and mucosal antibody profile, including IgE, and measurement of T-cell-mediated immune responses and of specific proinflammatory and immunoregulatory cytokines synthesized after exposure to the new ingredient. Specific IgE responses to allergens can be assessed by measuring serum IgE concentrations by the radioallergosorbent test for binding of IgE. These tests can be used as level 1 and level 2 assessments, as indicated in Table 6-8, based on the criteria specified by the committee. Specific level 2 assessments should be performed when there is evidence of adverse effects from the more general level 1 assessments.

However because allergic sensitization is a rare event, level 1 assessments in unselected infants may not have the power to detect such responses. The evaluation of subpopulations of infants selected for pre-existing allergies will also not be helpful because they will not be sensitized to the novel protein under consideration. Thus the potential allergenicity of such ingredients must be carefully evaluated in the preclinical studies described in Chapter 5.

TABLE 6-8 Available and Potential Tools for Assessment of Immnological and Allergic Outcomes

Tool	Assessment Level	Current Use in Assessment	Potential Value for Safety Endpoint Testing	
			Advantage	Disadvantage
Potential for antigenicity and immunogenicity	1	Yes	Screening to demonstrate the ability to induce an immune response	Nonspecific, does not predict potential for disease
Serologic evidence of prior exposure to ingredient				
Serum antibody	1	Yes	Provides specific evidence of prior exposure to specific ingredient; can differentiate between recent and past exposure	Does not predict potential for disease; requires peripheral blood samples
Cellular immunity	2	Yes	Sensitive marker for immunoregulatory vs proinflammatory immune response	Difficult to perform routinely; requires cellular or tissue sample; not standardized

Continued

TABLE 6-8 continued

Tool	Assessment Level	Current Use in Assessment	Potential Value for Safety Endpoint Testing	
			Advantage	Disadvantage
Homocytotropic response (including immunoglobulin E)	1	Yes	Strong correlation with disease and anaphylaxis, helpful tool for follow up of clinical course	Variable sensitivity and specificity
Mucosal immune responses				
Salivary immunoglobulin A, immunoglobulin E	2	Yes	Marker for mucosal immunity	Not available routinely
Cellular response	2	No	Can identify with more specificity Th1 vs Th2 helper T-cell responses	Not available routinely; may not predict potential for disease
Pre- and post-exposure cytokine and chemokine profiles	2	No	Marker for immunologic down regulation	Not available for routine use
Induction of tolerance	2	No	May be useful in treatment of allergic disorders	May not predict potential for disease
Change in mucosal environment				
Microflora	2	No	Simple and easy approach to identify potential effect of ingredient on microflora; useful for follow up for treatment modalities	May not predict potential for or existence of active disease
Inflammation	2	No	Provides histological evidence of disease	Requires mucosal tissues; spectrum of inflammation may vary considerably within different ingredients
Clinical evaluation				
Skin-prick test	1	Yes	Sensitive marker of primary exposure; easy to perform; does not require blood samples	May not predict potential for disease; false positive results with cross-reactive responses
Food elimination	1	Yes	Highly effective for specific diagnosis or treatment of disease	Effective elimination often difficult for ubiquitous ingredients; prolonged time lag before clinical effect and elimination
Food challenge	2	Yes	High specificity for diagnosis of ingredient-related disease	Risk of increased disease; possible (but rare) anaphylaxis on reintroduction of the ingredient

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

Sensitized cells release specific cytokine and chemokine mediators that can be quantified. These include substances such as histamine and tryptase, as well as markers of inflammation in the gastrointestinal tract. The latter may be of value in the evaluation of allergic response since inflammation is a risk factor for increased sensitization. Markers of intestinal inflammation include eosinophil cationic protein in serum and feces, α-1 antitrypsin, and tumor necrosis factor alpha (Majamaa et al., 1996). Skin prick and patch tests have good negative predictive value, but poor positive predictive value, and therefore are of more limited use in clinical testing.

As with other clinical studies, the most definitive clinical assessments are accomplished by double-blind, controlled trials. However investigators should consider that oral provocation of sensitive subjects could result in severe reactions and therefore study conditions should be carefully designed and controlled.

Assessing Endocrinological Activity

The endocrine system consists of multiple organs that secrete a wide variety of hormones that are responsible for maintaining the proper biochemical milieu of the body. Hormones are biochemicals that are secreted by the various glands (e.g., pituitary, thyroid, parathyroid, pancreas, adrenal, testes, and ovary) and act at other sites within the body. These hormones usually act by signaling biochemical reactions at cell membranes or intracellularly. Changes in endocrine function may either be intrinsic (e.g., a decrease in thyroid function because iodine is missing from the diet) or extrinsic because of chemicals (including nutrients) in the formula that may act as endocrine effectors or disruptors. Changes in hormone function may first be evident in growth changes. Most changes may take months or years to become evident; for example, sex hormone disruption may not be obvious until puberty.

Breastfeeding does not eliminate the concern for infant exposure to hormones. Oral contraceptive hormones are excreted in milk, and cohorts have been followed long term to ensure that the concentrations seen do not change the onset or course of puberty. Any substance added to an infant formula that may change secretion or function of growth or sex hormones may require follow-up through adolescence.

Exposure to endocrine disruptors in the environment may be brief in relation to the life span. Changes may thus be transient or not measurable. Some parameters that may show an immediate effect upon disruption of the endocrine function are thyroid-stimulating hormone (TSH), triiodothyronine (T_3), thyroxine blood glucose (T_4), blood calcium, phosphorus, and urine-specific gravity. Measurement of possible long-term effects include ovarian or testicular function, obesity, and pituitary function. Most endocrine measurements can be conducted by collecting either the blood or the urine. It is important to consider the age, sex, dietary status, body size, and medications during the interpretation of the numerical value of any clinical test for the endocrine system, especially during the gestational age. Level 1 screening should contain at least one test from all of the major substances, organs, and outcomes of the endocrine system. They include growth (e.g., insulin-like growth factor-1), the thyroid (TSH, T_3, T_4), the adrenals (cortisol, adrenocorticotropic hormone), the parathyroid (calcium, parathyroid hormone), antidiuretic hormone (urine osmolality), and glucose/insulin (Sperling, 1996). If there are any adverse events detected, this leads to a level 2 assessment, which includes immunoassays, binding proteins, and imaging techniques (e.g., body imaging, radionuclide imaging) (Sperling, 1996). A summary of the clinical endpoints for the endocrine system is provided in Table 6-9.

TABLE 6-9 Endocrine Clinical Endpoints

Function	Level 1[a]	Level 2[b]
Growth	IGF-1	IGFBP-3
Thyroid	TSH immunoassays, T_3, T_4	Binding proteins, radionuclide imaging
Adrenal	ACTH, cortisol	CRH, plasma rennin, plasma aldosterone, body imaging
Parathyroid	Calcium, PTH	Phosphorus
Antidiuretic hormone	Urine osmolality	Urine sodium excretion
Glucose/insulin	Plasma insulin, glucose concentration	

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.
[a]IGF-1 = insulin growth factor-1, TSH = thyroid-stimulating hormone, T_3 = triiodothyronine, T_4 = thyroxine, ACTH = adrenocorticotropic hormone, PTH = parathyroid hormone.
[b]IGFBP-3 = insulin growth factor-binding protein-3, CRH = corticotrophin-releasing hormone.
SOURCE: Sperling (1996).

DEVELOPMENTAL-BEHAVIORAL OUTCOMES

". . . subtle behavioral effects can appear well in advance of clear neurological dysfunction."

The Importance of Assessing Developmental-Behavioral Outcomes

There are a number of reasons why it is essential to include developmental-behavioral outcomes in future studies of the safety of ingredients new to infant formulas (see Figure 6-1, Box 6 and Figure 6-3). First, behavioral outcome measures are sensitive to exposure to toxic substances, particularly at low exposure levels. There are potential consequences to the infant of deviations from normal developmental pathways. Therefore when evaluating the addition of ingredients new to infant formulas, avoidance of type II errors (failure to detect a real effect) may be more critical then avoidance of type I errors (accepting a spurious finding as significant). In order to minimize the likelihood of failing to detect developmentally meaningful consequences associated with the addition of ingredients new to infant formulas, investigators must go beyond traditional toxicological and morphological assessments. As Weiss (1995) has argued, focusing only on easily observed physical malformations may seriously underestimate the actual impact of toxic exposure at either an individual or a population level. Supporting this position, evidence from a number of studies indicates that following exposure to toxins, subtle behavioral effects can appear well in advance of clear neurological dysfunction (Evangelista de Duffard and Duffard, 1996; Sobotka et al., 1996).

Inclusion of developmental-behavioral deficits may be particularly critical when investigating low-level exposure to toxins (Gaylor et al., 1998). It has been hypothesized that when multiple outcomes can be affected by exposure to toxic substances, behavioral outcomes may be among the most sensitive to toxic effects. When expressed in terms of *level of exposure* required to produce an effect, going from highest to lowest required level, the order of outcome sensitivity would be: *mortality* > *malformations* > *physical growth* > *behavior* (Vorhees, 1986). This hypothesized hierarchical ordering has been documented with regard to exposure in utero to vitamin A, salicylates, mercury, and alcohol (Adams, 1993; Jacobson and Jacobson, 2000). While the structural or functional domains affected by exposure to toxins will vary depending on the developmental course of different organ and functional systems (Shaheen, 1984), in the first year of life there is rapid development of these systems. What this means is that during the time period of maximum human exposure

to infant formulas, subtle, but important, developmental consequences may not be detected in nonhuman-based preclinical studies that primarily focus on toxicity or morphological changes and that may not include potentially more sensitive developmental-behavioral endpoints that are comparable with those assessed at the human level.

Second, developmental-behavioral measures can have long-term predictive value. Little direct evidence is available comparing the relative long-term consequences of outcomes such as physical malformations versus behavioral deviations. However it has been hypothesized that the long-term consequences of alterations in some components of behavioral development may be more critical than physical consequences in terms of affecting the individual's ability to adapt to environmental demands (Russell, 1992). For example, both facial changes and cognitive deficits are associated with fetal alcohol syndrome. The most likely candidate to influence the individual's ability to succeed in school would be the cognitive consequences of fetal alcohol syndrome (Vorhees, 1986).

A third reason to include developmental-behavioral outcomes in studies of the safety of ingredients new to infant formulas is that bidirectional brain-behavior links exist (e.g., brain development mediates changes in behavioral competence, but the child's interactions with his or her environment also can influence brain development). Although this report discusses neural and behavioral development separately from each other, these two areas of development are closely interlinked. Obviously changes in central nervous system (CNS) structure and function act as critical mediators for infant developmental-behavioral changes (Johnson, 2001; Lozoff et al., 1998; Nelson, 1994, 1995). However the converse is also true. There is increasing evidence showing that brain development can reflect changes in the child's environment (Greenough and Black, 1992; Nelson and Bloom, 1997; Schore, 1994). For example, systematic differences in infant brain electrical activity have been related to differences in rearing styles between depressed and nondepressed mothers (Dawson and Ashman, 2000). Environmental changes can be driven by changes in the child's behavioral patterns, as seen in studies showing systematic changes in maternal independence- and dependence-fostering behaviors as their infant shows increased levels of functional competence (Kindermann, 1993). This means that exposure to toxic substances that initially impact upon brain development will result in synergistic bidirectional influences upon brain and behavior.

Criteria for Including Developmental-Behavioral Outcome Measures

Outcome Domains

When developmental-behavioral outcomes have been used, much of the research in behavioral teratology and behavioral toxicology was designed to investigate whether there were cognitive deficits associated with exposure to potentially toxic substances, such as lead (e.g., Bellinger, 1995; Cory-Slechta, 1990). However it is important to recognize that development in the early years of life is characterized by changes across multiple domains (Masten and Coatsworth, 1998; Wachs, 1999). Because infant development is multifaceted, it is important to go beyond a relatively narrow focus on cognitive outcomes in order to fully evaluate the potential adverse effects of the addition of ingredients new to infant formulas (Struthers and Hansen, 1992; Vorhees, 1986). Normality of development in one developmental domain does not necessarily mean that there will be normality across all domains (Lester et al., 1995). At a minimum, assessment of the potential developmental-behavioral consequences of the addition of ingredients new to infant formulas should encompass outcomes in the domains of: (1) sensory and motor development, (2) cognitive development, (3) affect and temperament, and (4) neural integrity (see Table 6-10).

RECOMMENDATION: Assessment of clinical endpoints should include measurement of infant sensory-motor, cognitive, affectual, and neural function with instruments that follow recommended criteria.

Level of Assessment Required

Ideally the level and type of assessment utilized to evaluate the safety of an ingredient new to infant formulas would be driven by known links between the class of the added ingredient and the specific aspects of brain and behavioral development. Within this framework, knowledge of which and to what degree specific neural structures, processes, or behavioral functions were influenced by a given ingredient would tell the researcher which brain areas or processes and which behavioral functions should be tested to determine possible adverse consequences associated with the new ingredient. There is a solid body of knowledge on nutrient-brain-behavior links for a few substances, such as iron, and a growing body of knowledge on such links for polyunsaturated fatty acids (Rao and Georgieff, 2000). Unfortunately, for the most part, the knowledge is insufficient to base the choice of neural-behavioral measures on the empirical evidence alone. For the majority of new ingredients added to infant formulas, choice of the level of assessment and the domains assessed will require balancing the empirical evidence on substance-brain-behavior links with integration of results from the preclinical studies on a given substance, with knowledge of the long-term relevance of different outcome measures. To this end, the committee proposes a hierarchical set of criteria to determine the level of assessment that is needed in future studies of the potential developmental-behavioral-neural consequences of new ingredients added to infant formulas (see Figure 6-3).

Level 1 assessments are based on using behavioral and neural screening measures that can be easily administered using parental report or during a routine well-baby physical exam, which can be carried out as part of a clinical trial using a standardized examination protocol (Table 6-10). Because these measures are lower in sensitivity than the measures sufficient for use in the other levels described here, what appears to reflect normal development on these measures does not necessarily mean that an ingredient added to infant formulas is safe. Because of the lower sensitivity of level 1 assessment measures, more stringent criteria must be met to justify use of such measures. Hence level 1 assessments are recommended *only* if all of the following decision criteria occur:

- There is no existing evidence indicating a direct link between the new ingredient, metabolites, secondary effectors, or source and impairments in either neural or behavioral functions in infancy. In the absence of empirical evidence, there is no accepted theory that would suggest a plausible link between the new ingredient or new ingredient source and either neural or behavioral functions in infancy.
- There is no existing evidence for significant individual differences in susceptibility to the ingredient, metabolites, secondary effectors, or source (e.g., there is little evidence supporting a link between intake of food coloring or sugar and attention deficit disorder in children, but there is evidence that a small proportion of children with attention deficit disorder may have adverse reactions to food coloring or sugar that can accentuate their hyperactive-inattentive behavior [Christensen, 1996]).
- There are neither empirical nor theoretical links between the ingredient, metabolites, secondary effectors, or source and the functioning of other organ systems that might indirectly influence brain or behavioral development (e.g., given existing evidence on relations between nutrition and brain development [Rao and Georgieff, 2000], a new ingredient, metabolite,

secondary effector, or source that had an adverse influence on the digestive system could well result in subtle nutritional deficits that could in turn influence brain development).

- There is *no* evidence of adverse effects in preclinical studies, including adverse effects with potentially plausible alternative explanations (e.g., the effects are viewed as the result of random chance or the reviewers believe that there may be methodological or statistical problems in the studies). This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 1 assessments would not be warranted since adverse effects were found.

If all of the above criteria are met, then level 1 assessment instruments can be used in clinical studies of the safety of new ingredients added to infant formulas. Recommended screening instruments appropriate for level 1 neural and behavioral assessments in the first year of life are shown in Table 6-10. The screening instruments are low cost and, therefore, the committee recommends studying behavior and development from each of the domains listed in the table (visual development, audition, motor development, cognition, temperament, and neural integrity).

Level 2 and 3 assessments involve detailed measures of child function in major developmental areas. For level 2 assessments, one instrument is used for each area of function, and only a single assessment occasion is required. Three of the criteria discussed above are also involved in decisions as to whether level 2 assessments should be required. They are required when there is not a plausible direct empirical or theoretical link between the new ingredient or new ingredient source and impairments in either neural or behavioral functions in infancy, but *one or more* of the following criteria occur:

- Based on either existing evidence or accepted theory, there are plausible links between the ingredient and other organ systems that might indirectly influence neural or behavioral development (e.g., recombinant proteins that had an adverse influence on the digestive system or pre- or probiotics that changed the nature of intestinal flora/fauna, which could also influence digestive processes; in either case, one result could be subtle nutritional deficits that in turn could act to influence brain development).
- There is evidence of adverse effects in preclinical studies for organ systems that could influence neural or behavioral development. This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 2 assessments would be required since adverse effects did occur. For example, given current knowledge linking iron deficiency in infancy to adverse cognitive and neural development (Rao and Georgieff, 2000), recent evidence showing lower-than-predicted iron retention by young infants (Fomon et al., 2000) would be a preclinical adverse effect that would require use of at least level 2 assessment procedures in clinical trials involving new ingredients that either included iron or could influence iron absorption.
- There is existing evidence for significant individual differences in susceptibility to the ingredient.

Level 3 assessments also require detailed measures of child function in major developmental areas. In addition, if more than one recommended instrument is available for a given function, then a second recommended instrument that assesses either converging or complementary functions should be used on each assessment occasion. Assessment on at least two separate occasions is required within the first year. Level 3 assessments are required when the criteria for level 2 assessments are met and/or the following additional criterion occurs:

TABLE 6-10 Screening Measures Used in Level 1 Assessment of Infants Exposed to Formulas Containing New Ingredients

Function	Measure	Assessment Source	Nature of Deviation from Expected Development
Visual development	Visual attention to stimuli (Lester and Tronick, 2001)	Standardized medical examination carried out as part of a clinical trial	Infant not tracking or responding to visual stimuli (e.g., direction if gaze not follow moving visual stimuli)
Audition	1. Observation of infant orientation to sounds (Cobo-Lewis and Eilers, 2001) 2. Given documented links between hearing acuity and early language development (Cobo-Lewis and Eilers, 2001), an alternative measurement would be to assess the infant's acquisition of language landmarks in the first year, such as appearance of syllabic contours at around 3 mo, onset of babbling between 6–9 mo, and recognizing familiar words between 7–8 mo (Bloom, 1998; Posner, 2001)	Parent report or standardized medical examination carried out as part of a clinical trial	Infant not responding to auditory stimulation or showing language development that is below what would be expected for a infant at a given chronological age
Motor development	Age at which infant achieves motor landmarks like postural control, eye-hand coordination, sitting, crawling	Standardized medical examination carried out as part of a clinical trial	Degree of deviation from expected development can be assessed using standard norms such as those found in the Revised Bayley motor scales (1993)
Cognition	Norm-referenced parent report measures of developmental progress on adaptive behavior scales, such as the survey form of the Vineland Adaptive Behavior Scales (Sparrow et al., 1984)	Parent report or standardized medical examination carried out as part of a clinical trial	Level of developmental competence two or more standard deviations below the norm
Temperament	Short but validated parent report measure of infant temperament, such as the 24-item Infant Characteristics Questionnaire (Bates and Bayles, 1984)	Parent report or standardized medical examination carried out as part of a clinical trial	Infant rated as consistently expressing a high intense negative mood

Neural integrity	Neural screening exam assessing dimensions, such as cranial nerve function, sensory reactivity, age appropriate reflexes, and autonomic nervous system function, which can be administered by a pediatric nurse (Slota, 1983) or a more detailed clinical neural assessment administered by a physician (Herskowitz and Rosman, 1985)	Standardized medical examination carried out as part of a clinical trial	Clinicians judgment of infant's level of neural integrity
	Standardized brief neurological assessment instrument with cut-off points for normal versus abnormal neural functioning, for example, the Infant Neurological International Battery (INIB) (Ellison et al., 1985; Ellison, 1994) or the Neurologic Evaluation of the Newborn and the Infant (NENI) (Harris and Brady, 1986); or summary scores, for example, NICU Network Neurobehavioral Scale: (NNNS) (Lester and Tronick, 2001)	Standardized medical examination carried out as part of a clinical trial or as part of an overall screening battery in a research assessment during the first 6 wk after birth (NNNS) or across the first year (INIB, NENI)	Infant falls below global neural integrity cut-off score or cut-off score for specific neural function

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

• Based on either existing evidence or accepted theory, there is a plausible direct link between the new ingredient or new ingredient source and impairments in either neural or behavioral functions in infancy.

RECOMMENDATION: A hierarchy of three levels of clinical assessment should be implemented:

• **Level 1 assessments.** Developmental measures that are easily administered but low in sensitivity.

• **Level 2 assessments.** In-depth measures of infant functions in major developmental areas (single assessment for each area with one instrument).

• **Level 3 assessments.** In-depth measures of infant functions in major developmental areas (repeated assessments with multiple instruments).

Identifying Appropriate Measurement Instruments

There are a large number of potential instruments available to assess outcomes in the multiple domains in which development occurs during the first year of life. Therefore it is important to specify criteria that would allow the identification of measurement instruments that are more appropriate for use in level 2 and level 3 assessments of the potential developmental-behavioral consequences of the addition of new ingredients to infant formulas. Based on a review of the literature, the committee has identified seven criteria to rank the utility of instruments used to assess infant sensory-motor, cognitive, affectual, and neural function in level 2 and level 3 studies. The criteria are:

• *Age appropriateness*. Because infant intake of formulas is at a maximum during the first year of life, the committee recommends concurrent measures that can be administered during this time period.

• *Potential long-term consequences*. It is essential that assessment measures are not only reliable, but also that they either have predictive value for functional competence beyond the first year of life or are precursors for critical mediators of later functional competence. An example of the latter type of measure would involve the assessment of infant behaviors that interfere with the establishment of adequate caregiver-infant relations (Chasnoff et al., 1987).

• *Sensitivity*. An instrument may be appropriate for the first year of life and may have long-term predictive value, but still be relatively insensitive to exposure to toxic substances. Given the consequences to the developing infant, avoidance of type II errors (failure to detect a real effect) may be more critical then avoidance of type I errors when evaluating addition of new ingredients to infant formulas. To maximize the ability to detect adverse consequences, measures should demonstrate sensitivity to exposure to toxic substances during infancy. In recommending a sensitivity criterion, the committee fully recognizes that new ingredients for infant formulas will have been extensively evaluated during the preclinical trials, making it highly unlikely that substances that are clearly toxic will be tested in clinical trials. Thus the committee does not expect the assessment instruments described below to be used in clinical trials involving known toxic substances. However if an assessment instrument has not been shown to be sensitive to known toxins in previous research studies, it is unlikely that it will be sensitive to potentially subtle adverse effects of those new ingredients that are tested in clinical trials. As a result, the committee requires, where possible, that the

instruments used must have previously demonstrated the ability to detect adverse consequences associated with children's exposure to known toxins.

- *Brain-behavior links.* To maximize the ability to understand the mechanisms that underlie deleterious effects associated with the addition of ingredients new to infant formulas, outcome measures at the human level should have documented links to CNS structural and functional development. Knowledge of the brain-behavior link allows investigators to select which behavioral outcomes are most likely to be affected when toxins are known to impact on a given set of neural functions and which neural functions to select when toxins are known to impact on a specific set of behaviors (Jacobson and Jacobson, 2000).
- *Cross-species generalizability.* To maximize generalizability from preclinical nonhuman research to human clinical research, outcome measures used in human studies should have behavioral analogues at the nonhuman level. (See Table 5-11 for information on integrated tests across species.)
- *Function specificity.* When there is a choice of instruments it is desirable to avoid using global outcome measures that combine multiple outcome dimensions into a single score. The effects on a specific outcome dimension of exposure to a toxin may be lost when the score on this dimension is combined with scores on other nonaffected dimensions (Grandjean et al., 1996; McCall and Appelbaum, 1991). Alternatively, children exposed to different toxins may end up with the same global score, but may arrive at this score via different developmental paths, thus masking the differential impact of different toxins (Jacobson, 1998).
- *Ease of administration.* Developmental-behavioral measures used in the infancy period should be noninvasive and, all other criteria being satisfied, they should be relatively easy to administer.

Utilizing Appropriate Study Design

In addition to the criteria for instrument selection, a review of the research literature also indicated that certain requirements should be an essential part of any human study that examines the potential consequences of the addition of new ingredients to infant formulas. The committee recommends that the following four requirements be met.

- *Establish adequate statistical power.* As noted earlier, there is a critical need to avoid type II errors when evaluating safety. Investigators should document that there is sufficient statistical power to detect adverse effects for all studies in this area. The importance of establishing the adequacy of statistical power is illustrated in studies investigating the developmental-behavioral consequences of the addition of LC-PUFAs to infant formulas. In a recent review of this question, Wroble and colleagues (2002) cited seven studies involving term infants. The committee assessed the statistical power of these studies plus two additional studies with term infants that were not cited in this review. In computing statistical power, it was assumed that studies should be able to detect at least moderate effect sizes. The analysis indicated that the average level of statistical power across these nine studies was 0.56 (median power = 0.48, range across studies = 0.34–0.97). Not surprisingly, in their review Wroble and colleagues (2002) concluded that the evidence for developmental-behavioral benefits for term infants from adding LC-PUFAs to infant formulas was inconsistent at best. While adverse consequences of such additions were reported in two of the nine studies on which the committee did power calculations (Jensen et al., 1997; Scott et al., 1998), for the majority of studies reviewed statistical power was inadequate to detect either beneficial

or adverse consequences of the addition of LC-PUFAs to infant formulas. Demonstration that statistical power is sufficient to detect at least moderate-effect sizes is strongly recommended for all future clinical studies on the safety of the addition of new ingredients to infant formulas. Documentation of sufficient power is the responsibility of those seeking to add a new ingredient.

- *Avoid control of mediator variables*. A typical design strategy is to statistically control for confounding variables that may covary with the predictor (toxin) or the outcome variable (e.g., statistically control for the influence of sociodemographic covariates of lead exposure in infants). However it is critical to distinguish between a *confounder* and a *mediator* variable. Confounder variables are independent risk factors that occur at a higher level among those exposed to a toxic substance than those not exposed. In contrast, mediator variables are developmental risk factors whose occurrence is caused by exposure to a toxic substance (Neuspiel, 1995). Mediator variables covary with the predictor and the outcome variable because they are the mechanism through which the predictor influences the outcome. If mediating variables are statistically controlled, overcorrection may occur and a real risk effect may be missed (Jacobson and Jacobson, 1996). For example, prenatal alcohol exposure has been shown to influence infant temperament, which influences mother-child interaction, which influences child cognitive performance (O'Connor et al., 1993). In this case it would be a mistake to covary out either infant temperament or mother-infant interaction since these are mediators of the effects of prenatal alcohol exposure (for a recent review of design and statistical procedures used to assess the impact of mediator variables, see Shrout and Bolger, 2002).
- *Use measurement aggregation*. In developmental-behavioral studies not all measures of a construct have equal sensitivity. Therefore it is important to use converging multiple measures of the construct so a real link is not missed due to reliance on a single, less-sensitive measure.
- *Use repeated measures*. It is important to look at performance on outcome measures across time. This is partly because the impact of exposure to a toxin may vary depending on age level (neural maturation) of the infant (Shaheen, 1984). In addition, the sensitivity of various measures of the same dimension may vary depending on the age of the child (e.g., intramodal visual recognition memory is a sensitive predictor at 7 months of age but not at 12 months; crossmodal transfer, which taps the same cognitive dimension, but at a more complex level, is not a sensitive predictor at 7 months of age but is at 12 months (Rose et al., 1989).

Assessing Sensory and Motor Functions

Visual and Auditory Function

Instruments that could be used to evaluate infant sensory competencies in studies on the developmental effects of adding new ingredients to infant formulas are shown in Table 6-11. Even though the instruments described in the table have not been specifically linked to CNS development, sensory functions tapped by these instruments clearly have specific associations with brain structure and function. While preferential-looking procedures to test visual function and visual reinforcement audiometry to test auditory function have not been used in studies with infants exposed to toxins, they have been extensively used in clinical studies. These two procedures are thus recommended as being "state of the art" in the behavioral assessment of visual and auditory function. They could be easily combined with the func-

tional neurological measures described later in this section, such as brain stem-evoked response, to obtain converging measurements of infant sensory competence.

Motor Function

In contrast to the areas of visual and auditory assessment, where there are relatively few behavioral measures available in the first year of life, there are far more choices available for the measurement of early motor function. Also in contrast to assessment of sensory function, none of the motor measures can be described as being the "gold standard." A list of available measures for the assessment of infant motor function are shown in Table 6-12. Even though specific instruments described in this table have not been linked to CNS development, links between motor and brain development have been well established. Abnormal motor behavior has been reported as characteristic of infants exposed to toxins (Schneider et al., 1989), but studies linking such exposure to performance on the instruments described in Table 6-12 have been relatively rare. In the absence of more satisfactory measures, two instruments are provisionally recommended to assess adequate motor development in studies involving the safety of addition of new ingredients to infant formulas: the Alberta Infant Motor Scale and the Movement Assessment of Infants Scale. While the long-term predictive validity of these specific scales has yet to be established, early motor competence per se is an important precursor for later critical developmental functions, such as exploration, goal-directed behavior, and spatial learning (Bertenthal et al., 1984; Bushnell and Boudreau, 1993; Smitsman, 2001). In addition, evidence from several studies has indicated that these instruments are sensitive to early exposure of human infants to toxic substances.

Assessing Cognitive Development

The majority of behavioral teratology and behavioral toxicology research studies that assessed infant cognitive development as an endpoint utilized the Bayley Scales of Infant Development as an outcome measure (Jacobson and Jacobson, 2000; Kaltenbach and Finnegan, 1989). There are a variety of reasons to question the heavy reliance on the Bayley scales, including poor predictive validity of performance when administered to infants less than 2 years of age (Bendersky and Lewis, 2001; Colombo, 1993), and the fact that even in the revised version of the scale, the first-year mental scale is still heavily weighted with motor items. These problems are particularly critical given the fact that it is during the first year when infant formulas are most likely to be consumed. In addition, the earlier (Bayley, 1969) version of the Bayley scale, used in the majority of previous research on this topic, yields a global mental development score. One consequence of using a global score is that the significant impact of developmental risk factors on a specific cognitive dimension may be lost when the depressed score on the affected dimension is combined with scores on other nonaffected cognitive dimensions (Grandjean et al., 1996; Jacobson, 1998; McCall and Appelbaum, 1991). While the most recent revision of the Bayley scales does allow scoring of dimensional subscale scores, the heterogeneous factor structure of these subscales still increases the likelihood that a specific effect on a single cognitive dimension could well be attenuated (Bendersky and Lewis, 2001). Thus the global score problem has not been completely solved, even in the revised edition of this scale (Bendersky and Lewis, 2001).

The importance of going beyond global cognitive assessments in studies on the impact of potential developmental risk factors has been strengthened by both conceptual and empiri-

TABLE 6-11 Behavioral Measures Available to Test Sensory Functions in Clinical Studies on the Safety of New Additions to Infant Formulas

Description	Selection Criteria Met	Rating	Comments
Tests of visual function			
Preferential looking procedures under controlled conditions using stimuli with different levels of visual contrast to assess visual acuity and contrast sensitivity (Posner, 2001)	Can be administered during the first year (Mayer and Arendt, 2001) Documented links to central nervous system (CNS) structure or function (Slater, 2001) Analogous measures available at the nonhuman level (Banks and Salapatek, 1983; Jacobs and Blakemore, 1988) Assesses specific functions (Posner, 2001) Relative ease of administration (Mayer and Arendt, 2001)	Recommended for use in either level 2 or level 3 assessments	Current standard measure of preferential looking is the Teller Acuity Card Procedure (Teller et al., 1986) Early acuity deficits can have long-term consequences (Posner, 2001), but little evidence on the specific predictive validity of this procedure A lack of response may be due to infant fatigue or reduced attention and examiner experience essential for younger infants (Mayer and Arendt, 2001)
Tests of auditory function			
Visual reinforcement audiometry, where the child's head turning toward different sounds at different frequency levels are reinforced by an interesting visual stimulus (Moore et al., 1977)	Can be administered during the first year (Cobo-Lewis and Eilers, 2001) Documented links to CNS structure or function (Fernald, 2001) Assesses specific functions (Cobo-Lewis and Eilers, 2001) Relative ease of administration (Cobo-Lewis and Eilers, 2001)	Recommended for use in either level 2 or level 3 assessments	Can be taught quickly since the behavioral response is very clear and computers can be used to control the recording and reinforcements Early hearing problems have long-term consequences, but little evidence on the predictive value of this procedure per se Except under highly controlled laboratory conditions, best used with infants at or above 5 mo of age (Cobo-Lewis and Eilers, 2001)
Observer-based psychoacoustic procedures, based on the same principle as visual reinforcement audiometry except that observers utilize multiple, often subtle, cues to judge if an infant is orienting towards a sound stimulus (Olsho et al., 1987)	Can be administered during the first year (Cobo-Lewis and Eilers, 2001) Documented links to CNS structure or function (Fernald, 2001) Assesses specific functions (Cobo-Lewis and Eilers, 2001)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Can be used with infants as young as 2 mo of age, but requires specialized and extensive examiner training (Cobo-Lewis and Eilers, 2001)

Habituation procedures based on sounds being played when an infant displays a particular behavior, such as high intensity sucking; when the infant behavior declines a new sound is played and if the infant can discriminate, then the behavior should increase in frequency (Jusczyk, 1985)	Can be administered during the first year (Fernald, 2001) Assesses specific functions (Fernald, 2001)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Can be used with relatively young infants, but requires sophisticated equipment

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

TABLE 6-12 Behavioral Measures Available to Test Motor Functions in Clinical Studies on the Safety of New Additions to Infant Formulas

Description	Selection Criteria Met	Rating	Comments
Alberta Infant Motor Scale Norm-referenced, observational-based assessment of gross motor and postural development (Piper and Darrah, 1994)	Can be administered during the first year (Piper and Darrah, 1994) Has shown sensitivity to exposure to toxic substances during the first year (Fetters and Tronick, 1996) Documented links to central nervous system (CNS) structure or function (Tanner, 1970) Assesses specific functions (Piper and Darrah, 1994)	In the absence of more adequate measures, recommended as the best option available for use in either level 2 or level 3 studies	Range from birth–18 mo (Piper and Darrah, 1994); has satisfactory test-retest and interobserver reliability and discriminative validity; little assessment of fine motor skills Background in infant motor development important for valid observations
Movement Assessment of Infants Scale Assesses muscle tone, reflexes, and functional movement; 65 items with risk points for each item (Harris et al., 1984)	Can be administered during the first year (Case-Smith and Bigsby, 2001) Has shown sensitivity to exposure to toxic substances during the first year (Arendt et al., 1998; Fetters and Tronick, 1996) Documented links to CNS structure or function (Tanner, 1970) Assesses specific functions (Harris et al., 1984)	In the absence of more adequate measures, recommended as the best option available for level 2 or level 3 assessments	Range from birth–12 mo; inconsistent evidence on predictive validity (Case-Smith and Bigsby, 2001) Experience with infant motor development essential for valid administration (Chandler et al., 1980)
Revised Bayley Psychomotor Scale Revised version of earlier Bayley motor scale that assesses gross and fine motor skills (Bayley, 1993)	Can be administered during the first year (Bayley, 1993) Documented links to CNS structure or function (Tanner, 1970)	Meets few selection criteria; use only under limited or special circumstances	84% item overlap from 1969 version; better predictive validity for clinical than nonclinical populations (Bendersky and Lewis, 2001) Data inconsistent in regard to sensitivity of Bayley motor scale to exposure to toxic substances (e.g., Mayes and Cicchetti, 1995; Singer et al., 1997 vs. Hurt et. al., 1995; Jacobson et al., 1996)

Infant Neurological International Battery 20-item, criterion-referenced scale assessing muscle range, reflexes, and motor milestones (Ellison, 1994)	Can be administered during the first year (Case-Smith and Bigsby, 2001)	Meets few selection criteria; use only under limited or special circumstances	Range from birth–18 mo; satisfactory inter-rater, test-retest, and discriminative validity (Case-Smith and Bigsby, 2001)
Milani-Comparetti Development Screening Test-Revised 27-item, norm-referenced measure that assesses reflexes, postural control, and movement (Milani-Comparetti and Gidoni, 1967)	Can be administered during the first year (Milani-Comparetti and Gidoni, 1967) Documented links to CNS structure or function (Tanner, 1970)	Meets few selection criteria; use only under limited or special circumstances	Range 1–16 mo (Case-Smith and Bigsby, 2001); can be administered in a relatively brief period of time; satisfactory inter-rater and test-retest reliability
Test of Infant Motor Performance Consists of 27 observational items and 25 items that are elicited by the examiner; norm-referenced measure that assesses posture and movements (Campbell et al., 1993)	Can be administered during the first year (Campbell et al., 1993) Documented links to CNS structure or function (Tanner, 1970)	Meets few selection criteria; use only under limited or special circumstances	Restricted age range from birth–4 mo; satisfactory inter-rater reliability and internal consistency Requires highly trained examiner (Case-Smith and Bigsby, 2001)
Toddler and Infant Motor Evaluation Norm-referenced measure based on parent-elicited behaviors and observation; assesses motor mobility, stability, motor organization, and functional performance (Miller and Roid, 1994)	Can be administered during the first year (Miller and Roid, 1994) Documented links to CNS structure or function (Tanner, 1970)	Meets few selection criteria; use only under limited or special circumstances	Well standardized with satisfactory inter-rater and test-retest reliability and discriminative validity Requires skilled examiner and needs to be scored from videotape (Case-Smith and Bigsby, 2001)
Visually Guided Reaching A normal developmental function that shows an increase in amount and coordination from 4–8 mo; typically after 8 mo the infant no longer needs ongoing visual guidance for accurate reaching (Bushnell, 1985; von Hofsten, 1991)	Can be administered during the first year (Bushnell, 1985) Documented links to CNS structure or function (Johnson, 2001; von Hofsten and Fazel-Zandy, 1984) Analogous measures available at the nonhuman primate level (Johnson, 2001) Assesses specific functions (Bushnell, 1985)	Use only under limited or special circumstances	Not a formal test, but there are laboratory-based procedures to assess the changes in visually guided reaching over time Based on developmental norms; would expect increases in this behavior from 4–8 mo with decreases thereafter if the infant is developing normally

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

cal advances in the study of early cognitive development. Specifically, there is increasing agreement among developmental researchers that early intellectual development can be characterized by performance on three specific dimensions, which the committee recommends as the preferred dimensions of early cognitive performance for future clinical research studies. These are:

- Speed of processing, as assessed on tasks like habituation and visual expectancy performance (Colombo, 1993; Dougherty and Haith, 1997). Tasks of this type may be sensitive to nutritional intakes related to neural developmental processes involving myelination, synaptogenesis, and pruning (Rao and Georgieff, 2000).
- Recognition memory, as assessed on tasks like novelty preference and conjugate reinforcement (Fagen and Ohr, 2001; Rovee-Collier and Barr, 2001). Tasks of this type may be sensitive to intake of energy-glucose, iron, and zinc, which may relate to hippocampal development (Nelson et al., 2000; Pinero et al., 2001; Takeda, 2001).
- Behavioral inhibition, as assessed on measures like the A-not-B task (Fagen and Ohr, 2001; McCall, 1994). Tasks of this type may be sensitive to nutrients involved in monoamine synthesis (Lozoff et al., 1998).

Table 6-13 summarizes the available evidence that the committee reviewed in order to recommend these specific measures of cognitive function during the first year of life. While the table does not include an exhaustive list, it clearly illustrates the utility of assessment procedures that tap the three dimensions in studies on the potential cognitive consequences associated with the addition of new ingredients to infant formulas. Evidence suggests that toxic substances may differentially impact on different dimensions of cognitive performance (e.g., prenatal alcohol exposure is related to slower processing speed in infancy, but not to memory performance, and prenatal exposure to polychlorinated biphenyls adversely affects infant memory, but not processing speed [Jacobson, 1998]). This pattern of specificity may reflect differential sensitivity of the exposure of various brain areas to toxic substances, underlying different dimensions of early information processing (Jacobson and Jacobson, 2000; Mayes and Bornstein, 1995; Rao and Georgieff, 2000). The likely possibility of specificity of effects due to different potential toxins underlies the recommendation to assess at least two different dimensions of infant cognitive performance in level 3 studies on the cognitive impact of the addition of new ingredients to infant formulas.

Assessing Infant Temperament

Over the past 15 years there has been an increasing focus on noncognitive aspects of early development, with specific reference to the domain of infant temperament (Kohnstamm et al., 1989). Temperament refers to early-appearing, biologically rooted individual differences in behavioral tendencies that are relatively stable across contexts and time (Bates, 1989). The biological roots of individual differences in temperament include genetic influences (Goldsmith et al., 1994), exposure to toxic substances (Alessandri et al., 1995), and nutritional deficiencies (Wachs, 2000). While researchers have identified a number of different domains of infant temperament, recent formulations have indicated that these different domains converge on two major dimensions: reactivity and self-regulation (Rothbart and Bates, 1998). *Reactivity* refers to the speed, quality, and strength of the infant's reaction to stimulation (e.g., stimulus sensitivity, negative affect). *Self-regulation* refers to the quality and level of the infant's ability to control emotional responses (e.g., soothability, attentional allocation). Individual differences in temperament-driven behavioral patterns, like tenden-

cies towards approach and inhibition, are based on the balance between individual self-regulation and reactivity.

There have been two major approaches used to assess infant temperament. The first involves parent report measures using standardized scales. While parent report measures have been criticized for assessing parental emotional qualities rather than child temperament characteristics, reviews indicate that such measures are predictive in ways that would not occur if they were just measuring parental characteristics (Wachs and Bates, 2001). The second approach utilizes structured laboratory-based assessments of infant temperament. These assessments involve presenting a series of structured situations to an infant, videotaping the infant's behavior during the situations, and then coding the observed behaviors into temperament-related dimensions. Laboratory-based assessment procedures have been criticized for only sampling a restricted set of child behaviors. However the strengths of this approach include high intercoder reliability when using trained coders and the fact that these procedures do predict later developmental outcomes (Wachs and Bates, 2001).

At present, few of the standard parent report or laboratory-based assessment approaches for assessing infant temperament have been utilized in behavioral teratology or behavioral toxicology studies. However there are a number of reasons that support the need for use of such measures in future studies on questions involving the safety of new ingredients added to infant formulas. Specifically, evidence indicates that measures of temperament in the first year of life predict later personality (Rothbart et al., 2000), as well as attention and behavioral problems in preschool and school-age children (Bates, 2001; Posner, 2001; Robson and Pederson, 1997). The predictive function of early temperament problems may be partly due to an increased likelihood of problems in parent-child reactivity or with impairments in children's ability to self-regulate (Bendersky et al., 1996; O'Connor et al., 1993; Rossetti Ferreira, 1978; Rothbart and Bates, 1998; Schneider et al., 1989).

In addition, individual differences in early temperament have been linked to structural and functional development of the CNS (Nelson, 1994; Posner, 2001; Rothbart et al., 1994), and nonhuman analogues to human temperament measures have been reported (Higley and Suomi, 1989; Laughlin et al., 1991; Spear, 1995). Finally, and perhaps most critically, although standard temperament instruments have rarely been used in studies of human infants exposed to toxic substances, a number of studies from the behavioral toxicology and teratology literature have reported that such infants do show behavioral impairments that are clearly temperament driven, including problems in reactivity (e.g., increased irritability, hypo- or hyper-reactivity to stimuli [Hill et al., 1989; Lester et al., 1995]), and in self-regulation (e.g., poor state control, low consolability, and poor impulse control [Chasnoff et al., 1987; Mayes et al., 1995]). One consequence of toxin-driven differences in infant temperament is that infants exposed to toxic substances are harder to test and are less likely to complete testing due to temperament characteristics, such as high activity, high distractibility, high negative emotionality, hypersensitivity, or low consolability (Fagen and Ohr, 2001; Mayes et al., 1995; Struthers and Hansen, 1992). Infants who fail to complete testing due to temperament characteristics may be at increased risk for later developmental problems (Bathurst and Gottfried, 1987; Sebris et al., 1984).

Table 6-14 lists recommended indices of infant temperament for future studies of the safety of the addition of new ingredients to infant formulas. Based on available evidence, the committee strongly recommends using converging laboratory and parent report measures of temperament rather than just laboratory or just parent report measures. In addition, the committee recommends obtaining both mother and father reports for parent report measures (Wachs and Bates, 2001). Recommended parent report measures include the Infant Behavior Questionnaire or the Infant Characteristics Questionnaire. Either the LAB-TAB

TABLE 6-13 Measures Available to Test First-Year Cognitive Function in Clinical Studies on the Safety of New Additions to Infant Formulas

Description	Selection Criteria Met	Rating	Comments
Habituation Repeated presentation of a visual or auditory stimulus to an infant; observers code length of fixation to the stimulus and decline in attentional behavior with repeated presentations (Colombo, 1993)	Can be administered during the first year (Colombo, 1993). Has predictive value beyond the first year of life (McCall and Carriger, 1993; Ruff and Rothbart, 1996; Slater, 1997) Has shown sensitivity to exposure to toxic substances during the first year (Hill et al., 1989; Jacobson and Jacobson, 2000) Documented links to central nervous system (CNS) structure (Nelson, 1995) Assesses specific functions (Colombo, 1993) Relative ease of administration (Fagen and Ohr, 2001)	Recommended for use in either level 2 or level 3 assessments	The most sensitive predictors of later cognitive function are total looking time and duration of longest fixation, both of which decline with age (Fagen and Ohr, 2001); infants whose fixation times do not decline with age are showing slower processing speed and would be considered as being at risk (Colombo, 1993) Sensitivity of habituation procedure to toxic exposure may be higher in first several months of life (Fagen and Ohr, 2001)
Recognition memory Based on the preference of infants for novel stimuli; infants are familiarized with a stimulus and then the familiar stimulus is paired with a novel stimulus; over repeated trials with different stimuli, observers code whether the infant orients toward the familiar or the novel stimulus (Rose and Orlian, 2001). In intramodal tasks the familiar and novel stimuli are in the same modality; cross-modal transfer is a more complex task in that it involves the child being familiarized with the stimulus in one sensory modality (e.g., tactual) and tested in a different	Can be administered during the first year (Colombo, 1993) Has predictive value beyond the first year of life (Rose and Feldman, 1995; Rose et al., 1989, 1991; Slater, 1997) Has shown sensitivity to exposure to toxic substances during the first year (Jacobson et al., 1996; Rose and Orlian, 2001); Struthers and Hansen, 1992) Documented links to CNS structure (Johnson, 2001; Nelson, 1995) Analogous measures available at the nonhuman primate level (Grant-Webster et al., 1990; Rose and Orlian, 2001)	Recommended for use in either level 2 or level 3 assessments	Less than 53% preference for novel stimuli is used as the cut-off to distinguish at-risk from not-at-risk infants (Fagan et al., 1986; Jacobson et al., 1996) Intramodal memory tasks are a stronger predictor of later cognitive performance below 12 mo; crossmodal tasks are a stronger predictor at 12 mo (Rose and Feldman, 1995) Crossmodal transfer can be seen in infants as young as 6 mo, but is much more characteristic of infants who are closer to 12 mo (Rose and Orlian, 2001)

modality for novelty preference (e.g., visual) with the same familiar stimulus and a novel stimulus (Rose and Feldman, 1990)	Assesses specific functions (Colombo, 1993) Relative ease of administration (Rose and Orlian, 2001)		
Conjugate reinforcement learning tasks Infant actions (e.g., leg kicking, arm waving, hitting a lever) activate an interesting audio-visual stimulus (e.g., mobile moves, clown face lights up, toy train moves); by repeating this procedure across sessions, measures of speed of acquisition, level of retention, and speed of extinction can be obtained (Rovee-Collier and Barr, 2001)	Can be administered during the first year (Rovee-Collier and Barr, 2001) Has predictive value beyond the first year of life (Colombo, 1993; Fagen and Ohr, 2001) Has shown sensitivity to exposure to toxic substances during the first year (Alessandri et al., 1993) Documented links to CNS structure (Nelson, 1995) Assesses specific functions (Rovee-Collier and Barr, 2001) Relative ease of administration (Fagen and Ohr, 2001)	Not recommended for level 2 assessments; can be used as the alternate instrument for level 3 assessments	Speed of acquisition is not predictive of later intelligence, but retention of what has been learned is predictive (Fagen and Ohr, 2001) Tasks can be computer driven, which increases cost, but then requires little examiner training; other tasks are nonautomated but require a moderate amount of examiner training (Fagen and Ohr, 2001)
A-not-B task Infant sees an interesting object hidden at a specific location; after infant retrieves the object, infant then sees the object hidden at a different location; does the infant go to the location where he/she previously found the object hidden or the location where he/she last saw the object hidden; the task can be made more difficult by increasing the time between when the infant saw the object hidden and when he/she is allowed to search for it (Ruff and Rothbart, 1996)	Can be administered during the first year (Ruff and Rothbart, 1996) Documented links to CNS structure (Nelson, 1995; Posner, 2001) Analogous measures available at the nonhuman primate level (Diamond, 1990) Assesses specific functions (Ruff and Rothbart, 1996) Relative ease of administration (Ruff and Rothbart, 1996)	Not recommended for level 2 assessments; can be used as the alternate instrument for level 3 assessments	Past 6 mo of age, as infants get older, there is an increase in the amount of delay time infants can tolerate and still search successfully; older infants who search where the object was hidden only with no delay or who do not show increased tolerance for delay over time would be considered as being at risk (Ruff and Rothbart, 1996)

(continued on next page)

TABLE 6-13 continued

Description	Selection Criteria Met	Rating	Comments
Visual expectation Infant seated before a stimulus panel that has lights that flash on and off in a regular sequence; assesses whether child orients to the next light in the sequence before it comes on; can obtain measures of reaction time and number of correct anticipations (Colombo, 1993)	Can be administered during the first year (Colombo, 1993) Has predictive value beyond the first year of life (Dougherty and Haith, 1997) Assesses specific functions (Colombo, 1993)	Not recommended for level 2 assessments; can be used as the alternate instrument for level 3 assessments	Stimulus anticipation is a less-strong predictor of later cognitive performance than reaction time (Colombo, 1993) Visual expectation performance either does not distinguish between infants who were or were not exposed to toxic substances (Fagen and Ohr, 2001), or findings are inconsistent with regard to the question of whether exposed infants show slower or faster reaction times (Jacobson, 1998) Requires both sophisticated equipment and a high level of examiner training (Fagen and Ohr, 2001)
Focused attention Usually assessed during free play; using standardized rating criteria, observers code the amount of time the infant is attending to properties of the object they are playing with or exploring the object to see what can be done with it (Lawson and Ruff, 2001)	Can be administered during the first year (Ruff and Rothbart, 1996) Has predictive value beyond the first year of life (Lawson and Ruff, 2001) Documented links to CNS structure (Lawson and Ruff, 2001) Assesses specific functions (Ruff and Rothbart, 1996)	Not recommended for level 2 assessments; can be used as the alternate instrument for level 3 assessments	Infants showing less than 2% of time spent in focused attention during free-play task at 7 mo may be at developmental risk (Lawson and Ruff, 2001) Requires substantial training to learn to recognize focused attention and to maintain examiner calibration over time (Lawson and Ruff, 2001)
Bayley Mental Development Scale: 2nd ed. (Bayley, 1993)	Can be administered during the first year (Bayley, 1993)	Meets few selection criteria; use only under limited or special circumstances	Consistent evidence indicating low predictive validity of Bayley scale performance when administered below 2 y (Bendersky and Lewis, 2001; Colombo, 1993); inconsistency of findings when comparing Bayley mental development scores of infants

	exposed to toxic substances versus nonexposed control infants (e.g., see Arendt et al., 1998; O'Connor et al., 1993; Singer et al., 1997 vs. Hurt et al., 1995; Jacobson et al., 1996; Mayes and Cicchetti, 1995)
	Most recent revision of the Bayley scales does allow scoring of dimensional subscale scores, but the heterogeneous factor structure of these subscales still increases the likelihood that a specific effect on a single cognitive dimension could well be attenuated (Bendersky and Lewis, 2001)

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

or the Louisville Temperament Assessment Battery are recommended for laboratory-based measures.

Neurological Function

Knowledge of the associations among toxin, brain, and behavior increases the ability to detect the neural precursors of developmental-behavioral consequences of early exposure to toxic substances; knowledge of toxin-affected neural precursors helps to select which behavioral outcomes are most likely to be affected by exposure to toxins; and knowledge of brain-behavior relations can aid the researcher in selecting which areas of the brain to investigate in children who display specific developmental deficits as a result of exposure to toxic substances (Jacobson and Jacobson, 2000). With the explosion in neuroimaging techniques, there have been major advances in the study of brain metabolism and electrical activity as a window to CNS structure and function in children (Nelson and Bloom, 1997; Posner, 2001). Neuroimaging techniques can provide a more objective assessment of brain activity than can neurobehavioral measures. Further, many of these new techniques offer the promise of allowing researchers to detect relatively subtle neural deficits that may result from exposure to toxic substances.

Table 6-15 lists measures of CNS structure and function that may be useful in the study of developmental consequences of exposure to new ingredients in infant formulas. Electroencephalographic assessment and measures based on assessment of event-related potential are specifically recommended. While the instruments listed in Table 6-15 fit most of the selection criteria presented earlier, it is important to recognize that almost all of these instruments are expensive and require highly trained staff to operate them and to interpret their results. The committee has noted in this table which instruments are relatively less costly or require relatively lower staff expertise. It should also be noted that not all existing measures of CNS structure and function are listed in the table. Certain measures, such as positron emission tomography (Bookheimer, 2000), X-ray computed tomography (Singer, 2001) and assessment of homovanillic acid levels as a marker for dopamine status (Needlman et al., 1995), are invasive procedures and thus contraindicated for normal infants. Other potentially promising measures, such as magnetic diffusion tensor imaging (Posner, 2001), magnetic encephalography, and functional near-infrared spectroscopy (Nelson et al., 2002), have only recently been developed and more needs to be known before their utility in studies on neural consequences of the addition of new ingredients to infant formulas can be determined.

SUMMARY

Infancy is a particularly vulnerable period of development. A difference in growth is likely to signal some alteration in underlying biological or physiological processes. The committee took the conservative position that any systematic difference in growth that could be attributed to a new formula ingredient rather than to chance alone should represent a safety concern and should require explanation and further study. The major organ systems should be studied because growth deficits are likely to appear only secondary to effects on specific organs or tissues and may not appear for some time after nutritional insult. The committee therefore recommends implementing a hierarchy of two levels of clinical assessments for organ evaluations.

It is essential to include developmental-behavioral outcomes in future studies of the safety of ingredients new to infant formulas because such measures are sensitive to exposure

TABLE 6-14 Measures Available to Test First-Year Temperament in Clinical Studies on the Safety of New Additions to Infant Formulas

Description	Selection Criteria Met	Rating	Comments
Parent report measures			
Infant Behavior Questionnaire 87-item, parent report measure; can be administered starting at age 3 mo; assesses 5 dimensions of temperament (Rothbart, 1986)	Can be administered during the first year (Rothbart, 1986) Has shown sensitivity to exposure to toxic substances during the first year (Alessandri et al., 1995) Documented links to CNS structure (Goldsmith et al., 2000) Assesses specific functions (Rothbart and Bates, 1998)	Recommended for use in either level 2 or level 3 assessments	Infants rated as higher on negative affect, inhibition, and intensity (especially when linked to negative affect) and low on sociability, approach, positive affect, or soothability would be considered as being at greater risk for developmental problems. Relatively long measure and parents need to be literate in English
Infant Characteristics Questionnaire 24-item, parent report measure; can be administered starting at age 4 mo; assesses level of high-intense negative mood (Bates and Bayles, 1984)	Can be administered during the first year (Bates, 2001; Bates and Bayles, 1984) Assesses specific functions (Bates and Bayles, 1984) Relative ease of administration (Bates and Bayles, 1984)	Recommended for use in either level 2 or level 3 assessments	Infants rated as high on intense negative mood would be at greater risk for later developmental problems (Bates, 2001)
Revised Infant Temperament Questionnaire 95-item, parent report measure; can be administered starting at age 4 mo; assesses 9 dimensions of temperament (Carey and McDevitt, 1978)	Can be administered during the first year (Carey and McDevitt, 1978) Has predictive value beyond the first year of life (Sanson et al., 1996) Assesses specific functions (Martin et al., 1994)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Infants rated as higher on negative affect, inhibition, and intensity (especially when linked to negative affect) and low on sociability, approach, positive affect, or soothability would be considered as being at greater risk for developmental problems Relatively long measure and parents need to be literate in English

(continued on next page)

TABLE 6-14 continued

Description	Selection Criteria Met	Rating	Comments
Laboratory-based assessments			
LAB-TAB Laboratory-based temperament measure; assesses 5 dimensions of temperament, starting at age 6 mo (Goldsmith and Rothbart, 1991)	Can be administered during the first year (Goldsmith and Rothbart, 1991) Has predictive value beyond the first year of life (Rothbart et al., 2000) Assesses specific functions (Goldsmith and Rothbart, 1991)	Recommended for use in either level 2 or level 3 assessments	Infants rated as higher on negative affect, inhibition, and intensity (especially when linked to negative affect) and low on sociability, approach, positive affect, or soothability would be considered as being at greater risk for developmental problems Test requires a high level of examiner skill and coder competence
The Behavior Rating Scale on the 1993 Revision of the Bayley Scales of Infant Development-II Used to rate infant behavior during cognitive testing on 4 empirically derived factor scores, 3 of which involve temperament: attention/arousal, orientation/engagement, and emotional regulation (Bayley, 1993)	Can be administered during the first year (Bayley, 1993) Analogous measures available at the nonhuman primate level (Bayley, 1993)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Predictive validity has been demonstrated for the 1969 Bayley Infant Behavior Rating Scale (Matheny, 1989) Cut-off scores for each score have been developed, with scores below the 10th percentile being considered in the risk range (Bendersky and Lewis, 2001) Requires extensive examiner training (Bendersky and Lewis, 2001)
Louisville Temperament Assessment Battery Laboratory-based temperament measure; assesses 7 areas of temperament, starting at age 3 mo (Matheny, 1991)	Can be administered during the first year (Matheny, 1991) Has predictive value beyond the first year of life (Matheny and Phillips, 2001) Assesses specific functions (Matheny, 1991)	Recommended for use in either level 2 or level 3 assessments	Infants rated as higher on negative affect, inhibition and intensity (especially when linked to negative affect) and low on sociability, approach, positive affect, or soothability would be considered as being at greater risk for developmental problems Test requires high level of examiner competence and coder skill

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

TABLE 6-15 Measures Available to Test First-Year Neural Function in Clinical Studies on the Safety of the Addition of Ingredients New to Infant Formulas

Description	Selection Criteria Met	Rating	Comments
Event-related potential Assessment of latency and amplitude of electrical changes in different regions in the brain in response to specific sensory stimulation (Nelson and Bloom, 1997)	Can be administered during the first year (Posner, 2001) Has predictive value beyond the first year of life (Molfese and Molfese, 2001) Documented links to central nervous system (CNS) structure or function (Posner, 2001) Assesses specific functions (Nelson and Bloom, 1997)	Recommended for use in either level 2 or level 3 assessments	Noninvasive measure that is less sensitive to movement artifact then other neural measurements Requires substantial investment in equipment and examiner training (Posner, 2001) Spatial resolution is less adequate than temporal resolution, but still cannot assume 1 to 1 correspondence between brain electrical activity and what is recorded (Nelson et al., 2002) In contrast to evoked potentials, which assess the integrity of primary sensory pathways, measures appear to be assessing aspects of higher order cognitive processing such as attention
Electroencephalograph (EEG) Based on computerized analysis of electrical activity in different areas of the brain; of particular relevance are studies of relative electrical activity in different hemispheres of the brain (Marshall and Fox, 2001)	Can be administered during the first year (Marshall and Fox, 2001) Has predictive value beyond the first year of life (Fox et al., 2001) Has shown sensitivity to exposure to toxic substances during the first year (Kaneko et al., 1996; Needlman et al., 1995) Documented links to CNS structure or function (Posner, 2001) Analogous measures available at the nonhuman level (Needlman et al., 1995) Assesses specific functions (Marshall and Fox, 2001)	Recommended for use in either level 2 or level 3 assessments	Measures are noninvasive and are relatively inexpensive compared with other functional measures of the brain, but extensive training is required to avoid artifact and to interpret results correctly (Marshall and Fox, 2001) Cannot assume a 1 to 1 correspondence between brain electrical activity and what is recorded (Nelson et al., 2002)

(continued on next page)

TABLE 6-15 continued

Description	Selection Criteria Met	Rating	Comments
Cardiac variability-vagal tone Changes in heart rate as a function of stimulation are related to changes in respiratory sinus arrhythmia that reflect changes in the parasympathetic nervous system (Posner, 2001)	Can be administered during the first year (Porter, 2001) Has predictive value beyond the first year of life (Doussard-Roosevelt, et al., 2001; Porges et al., 1996) Has shown sensitivity to exposure to toxic substances during the first year (DiPietro et al., 1995) Documented links to CNS structure or function (Porter, 2001)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Individual differences in vagal tone have been linked to differences in attention and temperament, but this measure may primarily reflect general reactivity (Posner, 2001). Vagal tone has been shown to distinguish between breast- and formula-fed infants (DiPietro et al., 1987). Can be used in the first year, but lower stability early in the first year suggests this measure may be more useful after 6 mo (Porter, 2001) Requires extensive instrumentation and extensive training for valid interpretation of results (Porter, 2001)
Cortisol A hormonal measure based on the functioning of the hypothalamic-pituitary-adrenocortical axis; cortisol level can be viewed as the level of reactivity of the organism to stress (Gunnar, 2000)	Can be administered during the first year (Gunnar, 2000) Has shown sensitivity to exposure to toxic substances during the first year (Gunnar and White, 2001) Documented links to CNS structure or function (Gunnar, 2000) Analogous measures available at the nonhuman level (Needlman et al., 1995) Relative ease of administration (Gunnar and White, 2001)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Can be easily obtained from infant saliva, but since cortisol production follows a circadian rhythm and level of salivary cortisol can be influenced by recent intake of milk or milk products, assessment requires controls for time of day and milk exposure (Posner, 2001) Infants with consistently under- or overproduction of cortisol are considered as being at risk, but there are no specific norms as to what constitutes under- or overreaction (Gunnar and White, 2001); might be useful for assessing impact of new ingredients affecting sterol synthesis (including estrogen/phytoestrogen), along with its role as a stress marker

Structural magnetic resonance imaging Assesses size and changes in brain volume for different regions of the brain (Posner, 2001)	Can be administered during the first year (Singer, 2001) Has shown sensitivity to exposure to toxic substances during the first year (Mattson and Riley, 1995) Documented links to CNS structure or function (Posner, 2001) Analogous measures available at the nonhuman primate level (Hopkins and Rilling, 2000) Assesses specific functions (Posner, 2001)	Not recommended for level 2 assessments, but can be used as the alternate instrument for level 3 assessments	Excellent spatial resolution of brain structure, but requires a heavy investment in equipment and training (Posner, 2001) There would have to be a large impact of a new ingredient to reduce global or regional brain volume; an impact of this degree should have been detected in preclinical studies
Functional magnetic resonance imaging When a brain region is activated to deal with stimulation or task demands, there is increased blood and oxygen flow to that region; magnetic changes associated with increased hemoglobin flow to a specific brain region can be recorded as an index of increased activation of the region involved (Nelson and Bloom, 1997)	Documented links to CNS structure or function (Posner, 2001) Analogous measures available at the nonhuman primate level (Nakahara et al., 2002; Sereno, 1998) Assesses specific functions (Nelson and Bloom, 1997)	Meets few selection criteria; use only under limited or special circumstances	Because of the need to lie quietly and the high noise levels, it is not applicable for children under 6 y; however some recent studies using sedation of infants and passive presentation of stimulation have reported success with this procedure in infancy (Bookheimer, 2000) especially in napping postprandial babies (< 2 mo of age), but sedation would not be appropriate because of effects on cognitive processing (e.g., chloral hydrate)
Brain stem-evoked response EEG response of auditory brainstem responses to sound stimuli; allows assessment of the functional level of noncortical areas involved in hearing (Cobo-Lewis and Eilers, 2001)	Can be administered during the first year (Cobo-Lewis and Eilers, 2001) Has shown sensitivity to exposure to toxic substances during the first year (Needlman et al., 1995) Analogous measures available at the nonhuman level (Needlman et al., 1995) Assesses specific functions (Cobo-Lewis and Eilers, 2001)	Use only under limited or special circumstances	High number of false negatives and positives limit the utility (Molfese and Molfese, 2001) Does allow potential assessment of conduction speed of neural circuits involved in auditory processing (Roncagliolo et al., 1998)

NOTE: The petitioner (or manufacturer), in consultation with the expert panel, will determine which tests are required based on a thorough analysis of the potential effects of the new ingredient.

to toxic substances and can have long-term predictive value. These measures also are important because bidirectional brain-behavior links exist. In the case of neurological and behavioral assessment, the committee recommends that a hierarchy of three levels of clinical assessment be applied.

REFERENCES

AAP (American Academy of Pediatrics). 1988. *Clinical Testing of Infant Formulas with Respect to Nutritional Suitability for Term Infants. Report to the FDA*. Committee on Nutrition. Elk Grove Village, IL: AAP.

AAP. 1997. Breast feeding and the use of human milk. *Pediatrics* 100:1035–1039.

ADA (American Dietetic Association). 2001. Position of the American Dietetic Association: Breaking the barriers to breastfeeding. *J Am Diet Assoc* 101:1213–1220.

Adams J. 1993. Structure-activity and dose-response relationships in the neural and behavioral teratogenesis of retinoids. *Neurotoxicol Teratol* 15:193–202.

Alessandri SM, Sullivan MW, Imaizumi S, Lewis M. 1993. Learning and emotional responsivity in cocaine-exposed infants. *Dev Psychol* 29:989–997.

Alessandri SM, Sullivan MW, Bendersky M, Lewis M. 1995. Temperament in cocaine-exposed infants. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 273–286.

Antonowicz I, Lebenthal E. 1977. Developmental pattern of small intestinal enterokinase and disaccharidase activities in the human fetus. *Gastroenterology* 72:1299–1303.

Arant BS Jr, Edelmann CM Jr, Spitzer A. 1972. The congruence of creatinine and inulin clearances in children: Use of Technicon Autoanalyzer. *J Pediatr* 81:559–561.

Arendt R, Singer L, Angelopoulos J, Bass-Busdiecker O, Mascia J. 1998. Sensorimotor development in cocaine-exposed infants. *Infant Behav Dev* 21:627–640.

Armstrong J, Reilly JJ. 2002. Child Health Information Team. Breastfeeding and lowering the risk of childhood obesity. *Lancet* 359:2003–2004.

Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, Singer LT, Montalto MB, Jacobs JR, Qiu W, Bornstein MH. 2001. Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics* 108:372–381.

Auricchio S, Rubino A, Murset G. 1965. Intestinal glycosidase activities in the human embryo, fetus and newborn. *Pediatrics* 35:944–965.

Banks MS, Salapatek P. 1983. Infant visual perception. In: Mussen PH, ed. *Handbook of Child Psychology. Formerly Carmichael's Manual of Child Psychology*. 4th ed, vol 2. New York: John Wiley & Sons. Pp. 435–571.

Bates JE. 1989. Concepts and measures of temperament. In: Kohnstamm GA, Bates JE, Rothbart MK, eds. *Temperament in Childhood*. Chichester, Eng: John Wiley & Sons. Pp. 3–26.

Bates JE. 2001. Adjustment style in childhood as a product of parenting and temperament. In: Wachs TD, Kohnstamm GA, eds. *Temperament in Context*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 173–200.

Bates JE, Bayles K. 1984. Objective and subjective components in mothers' perceptions of their children from age 6 months to 3 years. *Merrill Palmer Q* 30:111–130.

Bathurst K, Gottfried AW. 1987. Untestable subjects in child development research: Developmental implications. *Child Dev* 58:1135–1144.

Batres LA, Maller ES. 2001. Laboratory assessment of liver function and injury in children. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. *Liver Disease in Children*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins. Pp. 155–169.

Bayley N. 1969. *Manual for the Bayley Scales of Infant Development*. New York: Psychological Corporation.

Bayley N. 1993. *The Bayley Scales of Infant Development*. 2nd ed. New York: Psychological Corporation.

Bellinger DC. 1995. Interpreting the literature on lead and child development: The neglected role of the "experimental system." *Neurotoxicol Teratol* 3:201–212.

Bendersky M, Lewis M. 2001. The Bayley scales of infant development. Is there a role in biobehavioral assessment? In: Singer LT Zeskind P, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 443–459.

Bendersky M, Alessandri SM, Lewis M. 1996. Emotions in cocaine-exposed infants. In: Lewis M, Sullivan MW, eds. *Emotional Development in Atypical Children*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 89–108.

Berenthal BI, Campos JJ, Barrett KC. 1984. Self-produced locomotion. In: Emde RN, Harmon RJ, eds. *Continuities and Discontinuities in Development*. New York: Plenum Press. Pp. 175–210.

Binley K, Askham Z, Iqball S, Spearman H, Martin L, de Alwis M, Thrasher AJ, Ali RR, Maxwell PH, Kingsman S, Naylor S. 2002. Long-term reversal of chronic anemia using a hypoxia-regulated erythropoietin gene therapy. *Blood* 100:2406–2413.

Bjarnason I, Macpherson A, Hollander D. 1995. Intestinal permeability: An overview. *Gastroenterology* 108:1566–1581.

Bloom L. 1998. Language acquisition in its developmental context. In: Kuhn D, Siegler RS, eds. *Handbook of Child Psychology*. 5th ed, vol 2. New York: John Wiley & Sons. Pp. 309–370.

Boehm G, Jakobsson I, Raiha N. 1992. Macromolecular absorption in small-for-gestational-age infants. *Acta Paediatr* 81:864–867.

Bookheimer SY. 2000. Methodological issues in pediatric neuroimaging. *Ment Retard Dev Disabil Res Rev* 6:161–165.

Brugnara C, Platt OS. 1998. The neonatal erythrocyte and its disorders. In: Nathan DG, Orkin SH, eds. *Nathan and Oski's Hematology of Infancy and Childhood*. 5th ed. Philadelphia: WB Saunders. Pp. 19–52.

Bushnell EW. 1985. The decline of visually guided reaching during infancy. *Infant Behav Dev* 8:139–155.

Bushnell EW, Boudreau JP. 1993. Motor development and the mind: The potential role of motor abilities as a determinant of aspects of perceptual development. *Child Dev* 64:1005–1021.

Butte NF. 2001. The role of breastfeeding in obesity. *Pediatr Clin North Am* 48:189–198.

Butte N, Heinz C, Hopkinhson J, Wong W, Shypailo R, Ellis K. 1999. Fat mass in infants and toddlers: Comparability of total body water, total body potassium, total body electrical conductivity, and dual-energy x-ray absorptiometry. *J Pediatr Gastroentrol Nutr* 29:184–189.

Butte NF, Wong WW, Hopkinson JM, O'Brian Smith E, Ellis KJ. 2000. Infant feeding mode affects early growth and body composition. *Pediatrics* 106:1355–1366.

Campbell SK, Osten ET, Kolobe THA, Fisher AG. 1993. Development of the test of infant motor performance. *Phys Med Rehabil Clin N Am* 4:541–550.

Canada. 2001. *Departmental Consolidation of the Food and Drugs Act and the Food and Drug Regulations (Food and Drugs Act)*. Ottawa: Minister of Public Works and Government Services Canada.

Carey WB, McDevitt SC. 1978. Revision of the infant temperament questionnaire. *Pediatrics* 61:735–739

Case-Smith J, Bigsby R. 2001. Motor assessment. In: Singer LT, Zeskind P, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 423–441

Chandler LS, Andrews MS, Swanson MW. 1980. *Movement Assessment of Infants. A Manual*. Rolling Bay, WA: Chandler, Andrews, and Swanson.

Chasnoff IJ, Burns KA, Burns WJ. 1987. Cocaine use in pregnancy: Perinatal morbidity and mortality. *Neurotoxicol Teratol* 9:291–293.

Christensen L. 1996. *Diet-Behavior Relationships: Focus on Depression*. Washington, DC: American Psychological Association.

Cobo-Lewis AB, Eilers R. 2001. Auditory assessment in infancy. In: Singer LT, Zeskind P, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 95–106.

Colombo J. 1993. *Infant Cognition: Predicting Later Intellectual Functioning*. Vol. 5. Newbury Park, CA: Sage Publications.

Cory-Slechta DA. 1990. Bridging experimental animal and human behavioral toxicology studies. In: Russell RW, Flattau PE, Pope AM, eds. *Behavioral Measures of Neurotoxicity. Report of a Symposium*. Washington, DC: National Academy Press. Pp. 137–158.

Cowin I, Emmett P. 2000. Cholesterol and triglyceride concentrations, birthweight and central obesity in pre-school children. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *Int J Obes Relat Metab Disord* 24:330–339.

Dawson G, Ashman SB. 2000. On the origins of a vulnerability to depression: The influence of the early social environment on the development of psychobiological systems related to risk for affective disorder. In: Nelson CA, ed. *The Minnesota Symposia on Child Psychology. Vol 31: The Effects of Early Adversity on Neurobehavioral Development*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 245–279.

Dewey KG, Heinig MJ, Nommsen LA, Lonnerdal B. 1991. Adequacy of energy intake among breast-fed infants in the DARLING study: Relationships to growth velocity, morbidity, and activity levels. Davis Area Research on Lactation, Infant Nutrition and Growth. *J Pediatr* 119:538–547.

Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. 1992. Growth of breast-fed and formula-fed infants from 0 to 18 months: The DARLING Study. *Pediatrics* 89:1035–1041.

Dewey KG, Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B. 1993. Breast fed infants are leaner than formula fed infants at 1 y of age: The DARLING study. *Am J Clin Nutr* 57:140–145.

Diamond A. 1990. The development and neural bases of memory functions as indexed by the AB and delayed response tasks in human infants and infant monkeys. *Ann N Y Acad Sci* 608:267–309.

Dietz WH. 1994. Critical periods in childhood for the development of obesity. *Am J Clin Nutr* 59:955–959.

Di Lorenzo C, Piepsz A, Ham H, Cadranel S. 1987. Gastric emptying with gastroesophageal reflux. *Arch Dis Child* 62:449–453.

Dinari G, Rosenbach Y, Zahavi I, Sivan Y, Nitzan M. 1984. Random fecal β_1-antitrypsin excretion in children with intestinal disorders. *Am J Dis Child* 138:971–973.

DiPietro JA, Larson SK, Porges SW. 1987. Behavioral and heart rate pattern differences between breast-fed and bottle-fed neonates. *Dev Psychol* 4:467–474.

DiPietro JA, Suess PE, Wheeler JS, Smouse PH, Newlin DB. 1995. Reactivity and regulation in cocaine-exposed neonates. *Infant Behav Dev* 18:407–414.

Dougherty TM, Haith MM. 1997. Infant expectations and reaction times as predictors of childhood speed and processing and IQ. *Dev Psychol* 33:146–155.

Doussard-Roosevelt JA, McClenny BD, Porges SW. 2001. Neonatal cardiac vagal tone and school-age developmental outcome in very low birth weight infants. *Dev Psychobiol* 38:56–66.

Dupont C, Barau E, Molkhou P, Raynaud F, Barbet JP, Dehennin L. 1989. Food-induced alterations of intestinal permeability in children with cow's milk-sensitive enteropathy and atopic dermatitis. *J Pediatr Gastroenterol Nutr* 8:459–465.

Ellison PH. 1994. *The INFANIB. A Reliable Method for the Neuromotor Assessment of Infants*. Tucson, AZ: Therapy Skill Builders.

Ellison PH, Horn JL, Browning CA. 1985. Construction of an Infant Neurological International Battery (INFANIB) for the assessment of neurological integrity in infancy. *Phys Ther* 65:1326–1331.

Esmon CT. 1998. Blood coagulation. In: Nathan DG, Orkin SH, eds. *Nathan and Oski's Hematology of Infancy and Childhood*. Philadelphia: WB Saunders. Pp. 1531–1556.

European Task Force on Atopic Dermatitis. 1993. Severity scoring of atopic dermatitis: The SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 186:23–31.

Evangelista de Duffard AM, Duffard R. 1996. Behavioral toxicology, risk assessment, and chlorinated hydrocarbons. *Environ Health Perspect* 104:353–360.

Fagan JF 3rd, Singer LT, Montie JE, Shepherd PA. 1986. Selective screening device for the early detection of normal or delayed cognitive development in infants at risk for later mental retardation. *Pediatrics* 78:1021–1026.

Fagen JW, Ohr PS. 2001. Learning and memory in infancy. Habituation, instrumental conditioning, and expectancy formation. In: Singer L, Zeskind P, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 233–273.

Falth-Magnusson K, Kjellman N-IM, Odelram H, Sundqvist T, Magnusson K-E. 1986. Gastrointestinal permeability in children with cow's milk allergy: Effect of milk challenge and sodium-cromoglycate as assessed with polyethyleneglycols (PEG 400 and 1000). *Clin Allergy* 16:543–551.

FDA (Food and Drug Administration). 1996. Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula; Proposed rule. *Fed Regist* 61:36153–36219.

Fee B, Weil WM. 1960. Body composition of a diabetic offspring by direct analysis. *Am J Dis Child* 100:718.

Fernald A. 2001. Hearing, listening, and understanding: Auditory development in infancy. In: Bremner G, Fogel A, eds. *Blackwell Handbook of Infant Development*. Malden, MA: Blackwell Publishers. Pp. 35–70.

Fernandes J, Vos CE, Douwes AC, Slotema E, Degenhart HJ. 1978. Respiratory hydrogen excretion as a parameter for lactose malabsorption in children. *Am J Clin Nutr* 31:597–602.

Fetters L, Tronick EZ. 1996. Neuromotor development of cocaine-exposed and control infants from birth through 15 months: Poor and poorer performance. *Pediatrics* 98:938–943.

Fomon SJ. 1993. *Nutrition of Normal Infants*. St. Louis: Mosby.

Fomon SJ, Ziegler EE, Thomas LN, Jensen RL, Filer LJ Jr. 1970. Excretion of fat by normal full-term infants fed various milks and formulas. *Am J Clin Nutr* 23:1299–1313.

Fomon SJ, Nelson SE, Ziegler EE. 2000. Retention of iron by infants. *Ann Rev Nutr* 1:273–290.

Forse RA, Bell SJ, Blackburn GL, Kabbash LG, eds. 1994. *Diet, Nutrition, and Immunity*. Boca Raton, FL: CRC Press.

Fox NA, Henderson HA, Rubin KH, Calkins SD, Schmidt LA. 2001. Continuity and discontinuity of behavioral inhibition and exuberance: Psychophysiological and behavioral influences across the first four years of life. *Child Dev* 72:1–21.

Fredrikzon B, Hernell O, Blackberg L, Olivecrona T. 1978. Bile salt-stimulated lipase in human milk: Evidence of activity in vivo and of a role in the digestion of milk retinal esters. *Pediatr Res* 12:1048–1052.

Garza C, de Onis M. 1999. A new international growth reference for young children. *Am J Clin Nutr* 70:169S–172S.

Gaylor DW, Bolger PM, Schwetz BA. 1998. U.S. Food and Drug Administration perspective of the inclusion of effects of low-level exposures in safety and risk assessment. *Environ Health Perspect* 106:391–394.

Gleim GW. 2000. Renal responses to exercise and training. In: Garrett WE, Krikendall DT, eds. *Exercise and Sport Science*. Philadelphia: Lippincott Williams & Wilkins. Pp. 217–225.

Godfrey KM, Barker DJ. 2001. Fetal programming and adult health. *Public Health Nutr* 4:611–624.

Goldsmith DI, Novello AC. 1992. Clinical laboratory evaluation of renal function. In: Edelmann CM, ed. *Pediatric Kidney Disease*. Boston: Little, Brown. P. 471.

Goldsmith HH, Rothbart MK. 1991. Contemporary instruments for assessing early temperament by questionnaire and in the laboratory. In: Strelau J, Angleitner A, eds. *Explorations in Temperament. International Perspectives on Theory and Measurement*. New York: Plenum Press. Pp. 249–272.

Goldsmith HH, Losoya SH, Bradshaw DL, Campos JJ. 1994. Genetics of personality: A twin study of the five-factor model and parent-offspring analysis. In: Halverson CF, Kohnstamm GA, Martin RP, eds. *The Developing Structure of Temperament and Personality from Infancy to Adulthood*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 241–265.

Goldsmith HH, Lemery KS, Aksan N, Buss KA. 2000. Temperamental substrates of personality development. In: Molfese VJ, Molfese DL, eds. *Temperament and Personality Development Across the Lifespan*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 1–32.

Grandjean P, White RF, Weihe P. 1996. Neurobehavioral epidemiology: Application in risk assessment. *Environ Health Perspect* 104:397–400.

Grant JD, Bezerra JA, Thompson SH, Lemen RJ, Koldovsky O, Udall JN. 1989. Assessment of lactose absorption by measurement of urinary galactose. *Gastroenterology* 97:895–899.

Grantham-McGregor SM, Walker SP, Chang S. 2000. Nutritional deficiencies and later behavioural development. *Proc Nutr Soc* 59:47–54.

Grant-Webster KS, Gunderson VM, Brubacher TM. 1990. Behavioral assessment of young nonhuman primates: Perceptual-cognitive development. *Neurotoxicol Teratol* 12:543–546.

Greenough WT, Black JE. 1992. Induction of brain structure by experience: Substrates for cognitive development. In: Gunnar MR, Nelson CA, eds. *Developmental Behavioral Neuroscience. The Minnesota Symposia on Child Psychology*. Vol. 24. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 155–200.

Gunnar MR. 2000. Early adversity and the development of stress reactivity and regulation. In: Nelson CA, ed. *The Effects of Early Adversity on Neurobehavioral Development. The Minnesota Symposia on Child Psychology*. Vol. 31. Mahwah, NJ: Lawrence Erlbaum Associates, Publishers. Pp. 163–200.

Gunnar MR, White BP. 2001. Salivary cortisol measures in infant and child assessment. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 167–189.

Guo SM, Roche AF, Fomon SJ, Nelson SE, Chumlea WC, Rogers RR, Baumgartner RN, Ziegler EE, Siervogel RM. 1991. Reference data on gains in weight and length during the first two years of life. *J Pediatr* 119:355–362.

Hadorn B, Zoppi G, Shmerling DH, Prader A, McIntyre I, Anderson CM. 1968. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 73:39–50.

Hamilton J. 2000. The pediatric patient: Early development and the digestive system. In: Walker WA, Durie PR, Hamilton JR, Walker-Smith JA, Watkins JB, eds. *Pediatric Gastrointestinal Disease*. 3rd ed. Hamilton, Ont: BC Decker. Pp. 1–11.

Handin RI. 1998. Blood platelets and the vessel wall. In: Nathan DG, Orkin SH, eds. *Nathan and Oski's Hematology of Infancy and Childhood*. Philadelphia: WB Saunders. Pp 1511–1529.

Harris SR, Brady DK. 1986. Infant neuromotor assessment instruments: A review. *Phys Occup Ther Pediatr* 6:121–153.

Harris SR, Swanson MW, Andrews MS, Sells CJ, Robinson NM, Bennett FC, Chandler LS. 1984. Predictive validity of the "movement assessment of infants." *J Dev Behav Pediatr* 5:336–342.

Hediger ML, Overpeck MD, Ruan WJ, Troendle JF. 2000. Early infant feeding and growth status of US-born infants and children aged 4–71 mo: Analyses from the Third National Health and Nutrition Examination Survey, 1988–1994. *Am J Clin Nutr* 72:159–167.

Herskowitz J, Rosman NP. 1985. Pseudoseizure in a child with epilepsy. *Am J Psychiatry* 142:390–391.

Heyman M, Grasset E, Ducroc R, Desjeux JF. 1988. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. *Pediatr Res* 24:197–202.

Higley JD, Suomi SJ. 1989. Temperamental reactivity in non-human primates. In: Kohnstamm GA, Bates JE, Rothbart MK, eds. *Temperament in Childhood*. Chichester, Eng: John Wiley & Sons. Pp. 153–167.

Hill RM, Hegemier S, Tennyson LM. 1989. The fetal alcohol syndrome: A multihandicapped child. *Neurotoxicology* 10:585–596.

Holm S, Andersson Y, Gothefors L, Lindbert T. 1992. Increased protein absorption after gastroenteritis in children. *Acta Paediatr* 81:585–588.

Holt PG, Macaubas C, Stumbles PA, Sly PD. 1999. The role of allergy in the development of asthma. *Nature* 402:B12–B17.

Hopkins WD, Rilling JK. 2000. A comparative MRI study of the relationship between neuroanatomical asymmetry and interhemispheric connectivity in primates: Implication for the evolution of functional asymmetries. *Behav Neurosci* 4:739–748.

Hurt H, Brodsky NL, Betancourt L, Braitman LE, Malmud E, Giannetta J. 1995. Cocaine-exposed children: Follow-up through 30 months. *J Dev Behav Pediatr* 16:29–35.

Huynh H, Couper R. 2000. Pancreatic function tests. In: Walker WA, Durie PR, Hamilton JR, Walker-Smith JA, Watkins JB, eds. *Pediatric Gastrointestinal Disease*. 3rd ed. Hamilton, Ont: BC Decker. Pp. 1515–1528.

Hypponen E, Virtanen SM, Kenward MG, Knip M, Akerblom HK. 2000. Obesity, increased linear growth, and risk of type 1 diabetes in children. *Diabetes Care* 23:1755–1760.

IOM (Institute of Medicine). 1991. *Nutrition During Lactation*. Washington, DC: National Academy Press.

IOM. 1997. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press.

IOM. 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academy Press.

Jacobs DS, Blakemore C. 1988. Factors limiting the postnatal development of visual acuity in the monkey. *Vision Res* 28:947–958.

Jacobson JL, Jacobson SW. 1996. Prospective, longitudinal assessment of developmental neurotoxicity. *Environ Health Perspect* 104:275–283.

Jacobson SW. 1998. Specificity of neurobehavioral outcomes associated with prenatal alcohol exposure. *Alcohol Clin Exp Res* 22:313–320.

Jacobson SW, Jacobson JL. 2000. Teratogenic insult and neurobehavioral function in infancy and childhood. In: Nelson CA, ed. *The Minnesota Symposia on Child Psychology. Vol 31: The Effects of Early Adversity on Neurobehavioral Development*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 61–112.

Jacobson SW, Jacobson JL, Sokol RJ, Martier SS, Chiodo LM. 1996. New evidence for neurobehavioral effects of in utero cocaine exposure. *J Pediatr* 129:581–590.

Jakobsson I, Lindberg T, Lothe L, Axelsson I, Benediktsson B. 1986. Human alpha-lactalbumin as a marker of macromolecular absorption. *Gut* 27:1029–1034.

Jensen C, Prager T, Fraley J, Chen H, Anderson R, Heird W. 1997. Effects of dietary linoleic/alpha-linolenic acid ratio or growth and visual function of term infants. *J Pediatr* 131:200–209.

Johnson MH. 2001. Functional brain development in humans. *Nat Rev Neurosci* 2:475–483.

Jusczyk PW. 1985. The high-amplitude sucking technique as a methodological tool in speech perception research. In: Gottlieb G, Krasnegor NA, eds. *Measurement of Audition and Vision in the First Year of Postnatal Life: A Methodological Overview*. Norwood, NJ: Ablex Publishing. Pp. 195–222.

Juvonen P, Jakobsson I, Lindberg T. 1990. Macromolecular absorption and cows milk allergy. *Arch Dis Child* 65:300–303.

Kaltenbach KA, Finnegan LP. 1989. Prenatal narcotic exposure: Perinatal and developmental effects. *Neurotoxicology* 10:597–604.

Kaneko WM, Phillips EL, Riley EP, Ehlers CL. 1996. EEG findings in Fetal Alcohol Syndrome and Down Syndrome children. *Electroencephalogr Clin Neurophysiol* 98:20–28.

Karpen SJ, Suchy FJ. 2001. Structural and functional development of the liver. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. *Liver Disease in Children*. Philadelphia: Lippincott Williams & Wilkins. Pp. 3–21.

Kay AB. 2001. Allergy and allergic diseases. First of two parts. *N Engl J Med* 344:30–37.

Kindermann TA. 1993. Fostering independence in mother-child interactions: Longitudinal changes in contingency patterns as children grow competent in developmental tasks. *Int J Behav Dev* 16:513–535.

Kohnstamm GA, Bates JE, Rothbart MK, eds. 1989. *Temperament in Childhood*. Chichester, Eng: John Wiley & Sons.

Kok EJ, Kuiper HA. 2003. Comparative safety assessment for biotech crops. *Trends Biotechnol* 21:439–444.

Koo WW. 2000. Body composition measurements during infancy. *Ann N Y Acad Sci* 904:383–392.

Kramer MS, Guo T, Platt RW, Shapiro S, Collet JP, Chalmers B, Hodnett E, Sevkovskaya Z, Dzikovich I, Vanilovich I. 2002. Breastfeeding and infant growth: Biology or bias? *Pediatrics* 110:343–347.

Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL. 2000. CDC growth charts: United States. *Adv Data* 314:1–27.

Laughlin NK, Bushnell PJ, Bowman RE. 1991. Lead exposure and diet: Differential effects on social development in the rhesus monkey. *Neurotoxicol Teratol* 13:429–440.

Lawson KR, Ruff HA. 2001. Focused attention: Assessing a fundamental cognitive process in infancy. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 3–17.

Leeson CP, Kattenhorn M, Deanfield JE, Lucas A. 2001. Duration of breast feeding and arterial distensibility in early adult life: Population based study. *Br Med J* 322:643–647.

Lester BM, Tronick EZ. 2001. Behavioral assessment scales. The NICU Network Neurobehavioral Scale, the Neonatal Behavioral Assessment Scale, and the Assessment of the Preterm Infant's Behavior. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 363–380.

Lester BM, Freier K, LaGasse L. 1995. Prenatal cocaine exposure and child outcome: What do we really know? In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 19–39.

Lindberg T. 1966. Intestinal dipeptidases: Characterization, development and distribution of intestinal dipeptidases of the human foetus. *Clin Sci* 30:505–515.

Lindsay RS, Cook V, Hanson RL, Salbe AD, Tataranni A, Knowler WC. 2002. Early excess weight gain of children in the Pima Indian population. *Pediatrics* 109:E33.

Lohman TG, Roche AF, Martorell R. 1988. *Anthropometric Standardization Reference Manual*. Champaign, IL: Human Kinetics Books.

Lothe L, Ivarsson SA, Ekman R, Lindberg T. 1990. Motilin and infantile colic. A prospective study. *Acta Paediatr Scand* 7:410–416.

Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. 1998. Behavior of infants with iron-deficiency anemia. *Child Dev* 69:24–36.

Maffei HV, Metz G, Bampoe V, Shiner M, Herman S, Brook CG. 1977. Lactose intolerance, detected by the hydrogen breath test, in infants and children with chronic diarrhoea. *Arch Dis Child* 52:766–767.

Majamaa H, Miettinen A, Laine S, Isolauri E. 1996. Intestinal inflammation in children with atopic eczema: Faecal eosinophil cationic protein and tumour necrosis factor-alpha as non-invasive indicators of food allergy. *Clin Exp Allergy* 26:181–187.

Marshall DH, Fox NA. 2001. Electroencephalographic assessment and human brain maturation. A window into emotional and cognitive development in infancy. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 341–356.

Martin RP, Wisenbaker J, Huttunen M. 1994. Review of factor analytic studies of temperament measures based on the Thomas-Chess structural model: Implications for the big five. In: Halverson CF Jr, Kohnstamm GA, eds. *The Developing Structure of Temperament and Personality from Infancy to Adulthood*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 157–172.

Masten AS, Coatsworth JD. 1998. The development of competence in favorable and unfavorable environments. Lessons from research on successful children. *Am Psychol* 53:205–220.

Matheny AP Jr. 1989. Temperament and cognition: Relations between temperament and mental test scores. In: Kohnstamm GA, Bates JE, Rothbart MK, eds. *Temperament in Childhood*. Chichester, Eng: John Wiley & Sons. Pp. 263–282.

Matheny AP Jr. 1991. Play assessment of infant temperament. In: Schaefer CE, Gitlin K, Sandgrund A, eds. *Play Diagnosis and Assessment*. New York: John Wiley & Sons. Pp. 39–63.

Matheny AP Jr, Phillips K. 2001. Temperament and context: Correlates of home environment with temperament continuity and change, new born to 30 months. In: Wachs TD, Kohnstamm GA, eds. *Temperament in Context*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 81–101.

Mattson SM, Riley EP. 1995. Prenatal exposure to alcohol. What the images reveal. *Alcohol Health Res World* 19:273–278.

Mayer DL, Arendt RE. 2001. Visual acuity assessment in infancy. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 81–94.

Mayes LC, Bornstein MH. 1995. Developmental dilemmas for cocaine-abusing parents and their children. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 251–272.

Mayes LC, Cicchetti DV. 1995. Prenatal cocaine exposure and neurobehavioral development: How subjects lost to follow-up bias study results. *Child Neuropsychol* 1:128–139.

Mayes LC, Bornstein MH, Chawarska K, Granger RH. 1995. Information processing and developmental assessments in 3-month-old infants exposed prenatally to cocaine. *Pediatrics* 95:539–545.

McCall RB. 1994. What process mediates predictions of childhood IQ from infant habituation and recognition memory? Speculations on the roles of inhibition and rate of information processing. *Intelligence* 18:107–125.

McCall RB, Appelbaum MI. 1991. Some issues of conducting secondary analyses. *Dev Psychol* 27:911–917.

McCall RB, Carriger MS. 1993. A meta-analysis of infant habituation and recognition memory performance as predictors of later IQ. *Child Dev* 64:57–79.

McMurray RG, Hackney AC. 2000. Endocrine responses to exercise and training. In: Garrett WE, Krikendall DT, eds. *Exercise and Sport Science*. Philadelphia: Lippincott Williams & Wilkins. Pp. 135–161.

Milani-Comparetti A, Gidoni EA. 1967. Routine developmental examination in normal and retarded children. *Dev Med Child Neurol* 9:631–638.

Miller LJ, Roid GH. 1994. *The T.I.M.E. Toddler and Infant Motor Evaluation. A Standardized Assessment*. San Antonio: Therapy Skill Builders.

Molfese DL, Molfese VJ. 2001. Cortical electrophysiology and language processes in infancy. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 323–338.

Montgomery RK, Mulberg AE, Grand RJ. 1999. Development of the human gastrointestinal tract: Twenty years of progress. *Gastroenterology* 116:702–731.

Moore JM, Wilson WR, Thompson G. 1977. Visual reinforcement of head-turn responses in infants under 12 months of age. *J Speech Hear Disord* 42:328–334.

Nakahara K, Hayashi T, Konishi S, Miyashita Y. 2002. Functional MRI of macaque monkeys performing a cognitive set-shifting task. *Science* 295:1532–1536.

Needlman R, Frank DA, Augustyn M, Zuckerman BS. 1995. Neurophysiological effects of prenatal cocaine exposure: Comparison of human and animal investigations. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 229–250.

Nelson CA. 1994. Neural bases of infant temperament. In: Bates JE, Wachs TD, eds. *Temperament: Individual Differences at the Interface of Biology and Behavior*. Washington, DC: American Psychological Association. Pp. 47–82.

Nelson CA. 1995. The ontogeny of human memory: A cognitive neuroscience perspective. *Dev Psychol* 31:723–738.

Nelson CA, Bloom FE. 1997. Child development and neuroscience. *Child Dev* 5:970–987.

Nelson CA, Wewerka S, Thomas KM, Tribby-Walbridge S, deRegnier R, Georgieff M. 2000. Neurocognitive sequelae of infants of diabetic mothers. *Behav Neurosci* 114:950–956.

Nelson CA, Bloom FE, Cameron JL, Amaral D, Dahl RE, Pine D. 2002. An integrative, multidisciplinary approach to the study of brain-behavior relations in the context of typical and atypical development. *Dev Psychopathol* 14:499–520.

Nelson SE, Rogers RR, Ziegler EE, Fomon SJ. 1989. Gain in weight and length during early infancy. *Early Hum Dev* 19:223–239.

Neuspiel DR. 1995. The problem of confounding in research on prenatal cocaine effects on behavior and development. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 95–109.

Norman A, Strandvik B, Ojamae O. 1972. Bile acids and pancreatic enzymes during absorption in the newborn. *Acta Paediat Scand* 61:571–576.

O'Connor MJ, Sigman M, Kasari C. 1993. Interactional model for the association among maternal alcohol use, mother-infant interaction, and infant cognitive development. *Infant Behav Dev* 16:177–192.

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS. 2003. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

Olsho LW, Koch EG, Halpin CF, Carter EA. 1987. An observer-based psychoacoustic procedure for use with young infants. *Dev Psychol* 23:627–640.

Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. 2000. Association between postnatal catch-up growth and obesity in childhood: Prospective cohort study. *Br Med J* 320:967–971.

Pelletier DL, Frongillo EA Jr, Habicht JP. 1993. Epidemiologic evidence for a potentiating effect of malnutrition on child mortality. *Am J Public Health* 83:1130–1133.

Perman JA, Barr RG, Watkins JB. 1978. Sucrose malabsorption in children: Noninvasive diagnosis by interval breath hydrogen determination. *J Pediatr* 93:17–22.

Pinero D, Jones B, Beard J. 2001. Variations in dietary iron alter behavior in developing rats. *J Nutr* 131:311–318.
Piper MC, Darrah J. 1994. *Alberta Infant Motor Scale: Construction of a Motor Assessment Tool for the Developing Infant*. Philadelphia: WB Saunders Company. Pp. 25–35.
Plancoulaine S, Charles MA, Lafay L, Tauber M, Thibult N, Borys JM, Eschwege E, Laventie Ville Sante Study Group. 2000. Infant-feeding patterns are related to blood cholesterol concentration in prepubertal children aged 5–11 y: The Fleurbaix-Laventie Ville Santé Study. *Eur J Clin Nutr* 54:114–119.
Porges SW, Doussard-Roosevelt JA, Portales AL, Greenspan SI. 1996. Infant regulation of the vagal 'brake' predicts child behavior problems: A psychobiological model of social behavior. *Devel Psychobiol* 29:697–712.
Porter FL. 2001. Vagal tone. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 109–124.
Posner MI. 2001. The developing human brain. *Dev Sci* 4:253–387.
Rao R, Georgieff MK. 2000. Early nutrition and brain development. In: Nelson C, ed. *The Minnesota Symposia on Child Psychology. The Effects of Adversity on Neurobehavioral Development*. Vol 31. Mahway, NJ: Lawrence Erlbaum Associates. Pp. 1–30.
Robb TA, Davidson GP. 1981. Advances in breath hydrogen quantitation in paediatrics: Sample collection and normalization to constant oxygen and nitrogen levels. *Clin Chim Acta* 111:281–285.
Roberton DM, Paganelli R, Dinwiddie R, Levinsky RJ. 1982. Milk antigen absorption in the preterm and term neonate. *Arch Dis Child* 57:369–372.
Robson AL, Pederson DR. 1997. Predictors of individual differences in attention among low birth weight children. *J Dev Behav Pediatr* 18:13–21.
Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. 1998. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: Delayed maturation of auditory brainstem responses. *Am J Clin Nutr* 68:683–690.
Rose SA, Feldman JF. 1990. Infant cognition: Individual differences and developmental continuities. In: Colombo J, Fagen J, eds. *Individual Differences in Infancy: Reliability, Stability, Prediction*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 229–245.
Rose SA, Feldman JF. 1995. Prediction of IQ and specific cognitive abilities at 11 years from infancy measures. *Dev Psychol* 31:685–696.
Rose SA, Orlian EK. 2001. Visual information processing. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 274–292.
Rose SA, Feldman JF, Wallace IF, McCarton C. 1989. Infant visual attention: Relation to birth status and developmental outcome during the first 5 years. *Dev Psychol* 25:560–576.
Rose SA, Feldman JF, Wallace IF, McCarton C. 1991. Information processing at 1 year: Relation to birth status and developmental outcome during the first 5 years. *Dev Psychol* 5:723–737.
Rossetti Ferreira MC. 1978. Malnutrition and mother-infant asynchrony: Slow mental development. *Int J Behav Dev* 1:207–219.
Rothbart MK. 1986. Longitudinal observation of infant temperament. *Dev Psychol* 22:356–365.
Rothbart MK, Bates JE. 1998. Temperament. In: Eisenberg N, ed. *Handbook of Child Psychology*. 5th ed, vol 3. New York: John Wiley & Sons. Pp. 105–176.
Rothbart MK, Derryberry D, Posner MI. 1994. A psychobiological approach to the development of temperament. In: Bates JE, Wachs TD, eds. *Temperament: Individual Differences at the Interface of Biology and Behavior*. Washington, DC: American Psychological Association. Pp. 83–116.
Rothbart MK, Derryberry D, Hershey K. 2000. Stability of temperament in childhood: Laboratory infant assessment to parent report at seven years. In: Molfese VJ, Molfese DL, eds. *Temperament and Personality Development Across the Life Span*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 85–118.
Rovee-Collier C, Barr R. 2001. Infant learning and memory. In: Bremner G, Fogel A, eds. *Blackwell Handbook of Infant Development*. Malden, MA: Blackwell Publishers. Pp. 139–168.
Ruff HA, Rothbart MK. 1996. *Attention in Early Development. Themes and Variations*. New York: Oxford University Press.
Russell RW. 1992. "Malnutrition" and the vulnerable brain. What a difference a molecule makes. In: Isaacson RL, Jensen KF, eds. *The Vulnerable Brain and Environmental Risks. Vol 1. Malnutrition and Hazard Assessment*. New York: Plenum Press. Pp. 109–126.
Sampson HA. 1996. Epidemiology of food allergy. *Pediatr Allergy Immunol* 7:42–50.
Sampson HA. 2000. Peanut allergy. *N Engl J Med* 346:1294–1299.
Sanderson IR, Walker WA. 1993. Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders (an update). *Gastroenterology* 104:622–639.

Sanson A, Pedlow R, Cann W, Prior M, Oberklaid F. 1996. Shyness ratings: Stability and correlates in early childhood. *Int J Behav Dev* 19:705–724.

Schmelzle HR, Fusch C. 2002. Body fat neonates and young infants: Validation of skinfold thickness versus dual-energy x-ray absorptimetry. *Am J Clin Nutr* 76:1096–1100.

Schneider JW, Griffith DR, Chasnoff IJ. 1989. Infants exposed to cocaine in utero: Implications for developmental assessment and intervention. *Infants Young Child* 2:25–36.

Schore AN. 1994. *Affect Regulation and the Origin of the Self. The Neurobiology of Emotional Development*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Schrander JJP, Unsalan-Hooyen RWM, Forget PP, Jansen J. 1990. ^{51}Cr EDTA intestinal permeability in children with cow's milk intolerance. *J Pediatr Gastroenterol Nutr* 10:189–192.

Scott DT, Janowsky JS, Carroll RE, Taylor JA, Auestad N, Montalto MB. 1998. Formula supplementation with long-chain polyunsaturated fatty acids: Are there developmental benefits? *Pediatrics* 102:1203–1204.

Scott RB. 2000. Motility disorders. In: Walker WA, Durie PR, Hamilton JR, Walker-Smith JA, Watkins JB, eds. *Pediatric Gastrointestinal Disease*. 3rd ed. Hamilton, Ont: BC Decker. Pp. 103–115.

Sebris SL, Dobson V, Hartmann EE. 1984. Assessment and prediction of visual acuity in 3- to 4-year-old children born prior to term. *Hum Neurobiol* 3:87–92.

Sereno MI. 1998. Brain mapping in animals and humans. *Curr Opin Neurobiol* 8:188–194.

Setchell KDR, O'Connell NC. 2001. Disorders of bile acid synthesis and metabolism: A metabolic basis for liver disease. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. *Liver Disease in Children*. Philadelphia: Lippincott Williams & Wilkins. Pp. 701–733.

Shaheen SJ. 1984. Neuromaturation and behavior development: The case of childhood lead poisoning. *Develop Psychol* 20:542–550.

Shrout PE, Bolger N. 2002. Mediation in experimental and nonexperimental studies. New procedures and recommendations. *Psychol Methods* 7:422–445.

Sicherer SH, Noone SA, Koerner CB, Christie L, Burks, AW, Sampson HA. 2001. Hypoallergenicity and efficacy of an amino acid based formula in children with cow's milk and multiple food hypersensitivities. *J Pediatr* 138:688–693.

Singer LT. 2001. General issues in infant assessment and development. In: Singer LT, Zeskind PS, eds. *Biobehavioral Assessment of the Infant*. New York: Guilford Press. Pp. 3–17.

Singer L, Arendt R, Farkas K, Minnes S, Huang J, Yamashita T. 1997. Relationship of prenatal cocaine exposure and maternal postpartum psychological distress to child developmental outcome. *Dev Psychopathol* 9:473–489.

Slater A. 1997. Can measures of infant habituation predict later intellectual ability? *Arch Dis Child* 77:474–476.

Slater A. 2001. Visual perception. In: Bremner G, Fogel A, eds. *Blackwell Handbook of Infant Development*. Malden, MA: Blackwell Publishers. Pp. 5–34.

Slota MC. 1983. Neurological assessment of the infant and toddler. *Crit Care Nurse* 3:87–92.

Smitsman MW. 2001. Action in infancy—Perspectives, concepts, and challenges: The development of reaching and grasping. In: Bremner G, Fogel A, eds. *Blackwell Handbook of Infant Development*. Malden, MA: Blackwell Publishers. Pp. 71–98.

Sobotka TJ, Ekelman KB, Slikker W Jr, Raffaele K, Hattan DG. 1996. Food and Drug Administration proposed guidelines for neurotoxicological testing of food chemicals. *Neurotoxicology* 17:825–836.

Sparrow SS, Balla DA, Cicchetti DV. 1984. *Vineland Adaptive Behavioral Scales*. Circle Pines, MN: American Guidance Service.

Spear LP. 1995. Alterations in cognitive function following prenatal cocaine exposure: Studies in an animal model. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 207–227.

Sperling MA. 1996. *Pediatric Endocrinology*. Philadelphia: WB Saunders. Pp. 549–584.

Stettler N, Bovert P, Shamlaye H, Zemel BS, Stallings VA, Paccaud F. 2002a. Prevalence and risk factors for overweight and obesity in children from Seycheles, a country in rapid transition: The importance of early growth. *Int J Obes Relat Metab Disord* 26:214–219.

Stettler N, Zemel BS, Kumanyika S, Stallings VA. 2002b. Infant weight gain and childhood overweight status in a multicenter, cohort study. *Pediatrics* 109:194–199.

Struthers JM, Hansen RL. 1992. Visual recognition memory in drug-exposed infants. *J Dev Behav Pediatr* 13:108–111.

Takeda A. 2001. Zinc homeostasis and functions of zinc in the brain. *Biometals* 14:343–351.

Tanner JM. 1970. Physical growth. In: Mussen PH, ed. *Carmichael's Manual of Child Psychology*. 3rd ed, vol 1. New York: John Wiley & Sons. Pp. 77–155.

Teller DY, McDonald MA, Preston K, Sebris SL, Dobson V. 1986. Assessment of visual acuity in infants and children: The acuity card procedure. *Dev Med Child Neurol* 28:779–789.

Thomas DW, Sinatra FR, Merritt RJ. 1981. Random fecal alpha-1-antitrypsin concentration in children with gastrointestinal disease. *Gastroenterology* 80:776–782.

van de Kamer JH, ten Bokkel Huinink H, Weyers HA. 1949. Rapid method for the determination of fat in feces. *J Biol Chem* 177:347–355.

Vijayakumar M, Fall CH, Osmond C, Barker DJ. 1995. Birth weight, weight at one year, and left ventricular mass in adult life. *Br Heart J* 73:363–367.

von Hofsten C. 1991. Structuring of early reaching movements: A longitudinal study. *J Mot Behav* 23:280–292.

von Hofsten C, Fazel-Zandy S. 1984. Development of visually guided hand orientation in reaching. *J Exp Child Psychol* 38:208–219.

Vorhees CV. 1986. Principles of behavioral teratology. In: Riley EP, Vorhees CV, eds. *Handbook of Behavioral Teratology*. New York: Plenum Press. Pp. 23–48.

Wachs TD. 1999. The nature and nurture of child development. *Food Nutr Bull* 20:7–22.

Wachs TD. 2000. Linking nutrition and temperament. In: Molfese VJ, Molfese DL, eds. *Temperament and Personality Development Across the Life Span*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 57–84.

Wachs TD, Bates JE. 2001. Temperament. In: Bremner G, Fogel A, eds. *Blackwell Handbook of Infant Development*. Malden, MA: Blackwell Publishers. Pp. 465–501.

Walker WA. 2002. Development of the intestinal mucosal barrier. *J Pediatr Gastroenterol Nutr* 34:S33–S39.

Watkins JB. 1985. Lipid digestion and absorption. *Pediatrics* 75:151–156.

Watkins JB, Ingall D, Szczepanik P, Klein PD, Lester R. 1973. Bile-salt metabolism in the newborn. Measurement of pool size and synthesis by stable isotope technique. *New Engl J Med* 288:431–434.

Weiss B. 1995. Incipient hazards of cocaine: Lessons from environmental toxicology. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 41–55.

Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. 1997. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med* 337:869–873.

WHO (World Health Organization). 2002. *The Optimal Duration of Exclusive Breastfeeding. Report of an Expert Consultation*. Geneva: WHO.

Wroble M, Mash C, Williams L, McCall R. 2002. Should long chain polyunsaturated fatty acids be added to infant formula to promote development? *J App Dev Psychol* 23:99–112.

Yajnik CS. 2001. The insulin resistance epidemic in India: Fetal origins, later lifestyle, or both? *Nutr Rev* 59:1–9.

7

Selecting an In-Market Surveillance Plan

ABSTRACT

In-market surveillance is an essential element of regulating the safety of ingredients new to infant formulas and should be included in new safety assessment guidelines. There are two components of in-market surveillance. The first, the "monitoring component," involves procedures to detect adverse effects upon infants after a formula has been put on the market. The second, the "follow-up component," concentrates on possible long-term adverse effects after the period of maximum formula usage. Although formal regulatory guidelines for in-market surveillance do not exist in the United States or Canada, infant formula manufacturers routinely conduct passive surveillance via toll-free calls, contact with health care professionals, and reports from their field sales force.

Satisfactory completion of the appropriate preclinical and clinical studies diminishes the likelihood of systematic adverse reactions; however the risks for adverse reactions cannot be ignored because adverse effects may not be detected in preclinical studies if the wrong animal model was chosen, if the assessment instrument chosen measured a function other than the one adversely affected by the new ingredient, or if a subpopulation of individuals who are highly sensitive to the new ingredient added to infant formula was not sufficiently represented in clinical studies.

The committee believes that there is a crucial need for follow-up strategies to ensure safety and normal development of the infant population (e.g., brain areas that are adversely affected by new ingredients may not functionally become apparent until later in development, or early exposure to a toxin may increase susceptibility to later exposure to toxins). The committee recommends that a systematic plan for continued in-market monitoring and long-term surveillance become an essential part of all submissions for regulatory agency review seeking to add a new ingredient to infant formula. The in-market surveillance strategies the committee proposes are specifically designed for evaluation of the safety of new ingredients added to infant

formulas and do not necessarily apply to the safety of new ingredients not intended for infant formulas.

Infant formula manufacturers should select in-market strategies by implementing a hierarchy of three levels of assessment, including passive surveillance (level 1 assessment), expert panel reviews of the literature (level 2 assessment), and active surveillance (level 3 assessment). Active surveillance is the most complex assessment and includes options such as studying specific populations, conducting retrospective studies, using the Pediatric Research in Office Settings program of the American Academy of Pediatrics, and conducting clinical follow-up studies of the original study populations. Selection of the appropriate level of assessment is based upon conditions under which potential adverse effects of a new ingredient added to infant formulas might have been missed in preclinical or clinical studies. The length of the follow-up period will depend on the targeted area (organ system), preclinical and clinical studies, and the nature of the added ingredient.

BACKGROUND

The Importance of Systematic In-Market Surveillance

Satisfactory completion of the appropriate preclinical and clinical studies diminishes the likelihood of adverse reactions to infant formulas that contain new ingredients once they have been placed on the market. However completion of those studies does not ensure absolute safety, and the probability of adverse reactions cannot be overlooked for several reasons.

First, adverse effects may not be detected in preclinical studies if the wrong animal model is chosen to map onto a function at the human level (see Chapter 5). Second, in clinical studies a less-sensitive instrument may fail to detect an adverse reaction due to poor initial outcome measurement (Clarke and Clarke, 1981; see Chapter 6). Third, adverse effects could be missed during clinical studies if the instrument chosen, even if highly sensitive, was measuring a function other than the one adversely affected by the new ingredient. A similar point holds in regard to predictor measures. Analytical procedures have been developed that are capable of measuring compounds at concentrations as low as picograms per liter. Concurrent appreciation of potential biological changes has not kept pace with this degree of laboratory sophistication. Finally, the sampling design of clinical trials could have been inappropriate. Given what is known about variability in individual or subpopulation sensitivities to exposure to dietary ingredients (Beaton, 1986; Rutter and Pickles, 1991) or toxic substances (Bellinger, 1995; Ruff, 1999), if a subpopulation of individuals who are highly sensitive to the new ingredient added to an infant formula are not sufficiently represented in the clinical studies, adverse effects may not be detected until the formula is marketed to a wider population.

These reasons support the need for a systematic plan to include both types of in-market surveillance into every submission to the regulatory agency for the addition of an ingredient new to infant formulas. The two types of in-market surveillance are discussed below.

RECOMMENDATION: A systematic plan for continued in-market monitoring and long-term surveillance should be an essential part of submissions for regulatory agency review in assessing the safety of ingredients new to infant formulas.

Monitoring

The first component of in-market surveillance, the monitoring component, involves procedures to detect adverse effects to infants after a formula has been put on the market. As discussed above, the probability that negative effects will emerge during in-market monitoring is likely to be quite small if the appropriate preclinical and clinical studies detected no negative effects associated with the introduction of a new ingredient to an existing formula. However the number of infants enrolled in clinical trials is small in relation to in-market use, and the trials may not detect a full range of variations. For this reason it is important to integrate in-market monitoring procedures into the evaluation process to judge the safety of new ingredients introduced into infant formulas.

Follow-up

The second component of in-market surveillance, the follow-up component, is one that is less often considered during clinical trials to assess the safety of new ingredients added to infant formulas. In contrast to in-market monitoring, which focuses on adverse effects occurring during the period of maximum formula usage, in-market follow-up concentrates on possible long-term adverse effects after the period of maximum formula usage. The length of a follow-up study will depend upon the nature of the ingredient, so the expert panel should define it on a case-by-case basis. As described later in this chapter, higher levels of assessments should be performed when the ingredient may affect slow-developing brain regions, hormone or neurotransmitter function, or behavior. In addition, follow-up during critical life transitions, such as entry into school or onset of puberty, should be emphasized.

Most clinical studies are confined to a short amount of time for the period of maximum exposure to the formulas, and they track adverse patterns only during the time of maximum exposure. However there is also the possibility that the new ingredient's negative effects on the growth and development of children may have delayed onset and only appear later in life. Evidence from a number of sources supports the validity of this statement. First, there are both preclinical and clinical studies that document long-term cognitive and behavioral effects of early exposure to toxic substances (Galler and Tonkiss, 1998; Jacobson and Jacobson, 2000; Leech et al., 1999; Leviton et al., 1993; Richardson, 1998; Richardson et al., 1996; Romano and Harvey, 1998; Wasserman et al., 2000).

More critically, although the overall pattern of evidence is not totally consistent, there are a number of examples from both the clinical and preclinical research literature where negative effects of early exposure to toxic substances were not found upon initial testing, but did appear during follow-up assessment (Weiss, 1995; Winneke, 1990). Toxic substances that do not show their effects until well after the period of exposure to the substance have been labeled as chemical or neurobehavioral "time bombs" (Russell, 1990; Spencer, 1990). Delayed effects have been shown for prenatal or early postnatal exposure to drugs, alcohol (Griffith et al., 1994; Singer et al., 2002), and lead (Bellinger et al., 1991). Although not directly related to toxic exposure, epidemiological studies have also indicated that there may be delayed long-term adult biomedical consequences as a result of the quality of very early nutrition (Barker et al., 1993; Jackson, 2000) or of the level of morbidity in the first year of life (Bengtsson and Lindstrom, 2000).

There are a number of mechanisms through which a delayed impact of early exposure to toxic substances might occur. One involves cumulative effects. Biologically, cumulating intake of a low-level toxin can result in the gradual replacement of a specific neurotransmit-

ter by a less efficient molecular substitute; only over time will the detrimental impact of the substitute become manifest (Russell, 1990). Another example is the long-chain polyunsaturated fatty acids (LC-PUFAs) that have been determined as GRAS for addition to infant formulas. These LC-PUFAs are derived from genetically selected algae that produce triglycerides with a high content of either arachidonic acid or docosahexaenoic acid onto two or three of the fatty acid chains of the triacylglycerol molecule. This contrasts to the triacylglycerol makeup of human milk, where LC-PUFAs rarely form two-thirds of the fatty acids of a single triacylglycerol molecule. In this case, diglycerides resulting from hydrolysis of the algae triglycerides in infant formula would be different than those produced from human milk and different biological effects could occur (e.g., specific diglycerides have very different effects in cell signaling pathways and could have different "triggering" effects on certain cellular pathways).

Such a cumulative effect scenario is particularly likely to occur when a toxic substance is contained in formula, which may be the main nutrient source for an infant over an extended period of time. A cumulative effect process, wherein initial small deficits cumulate and become significant only over time, appears to be a particularly likely scenario when the critical mechanisms are biobehavioral in nature (Wachs, 2000). Such a process could occur when toxic exposure leads to changes in infant temperament characteristics, such as an increased level of irritability or a reduced level of responsivity. Toxin-driven changes in temperament could adversely impact upon critical influences on later development, such as patterns of parent-child relations, which over time can lead to long-term developmental-behavioral deficits (Bendersky et al., 1996; Chasnoff et al., 1987; Fried, 1989; O'Connor et al., 1993; Schneider et al., 1999).

Alternatively, the area of the brain that is damaged by a toxic substance may be one in which the functions mediated by this area become apparent only later in development, or it could be one in which connections go from a damaged area to a later-developing area (Lyon and Gadisseux, 1991; Weiss, 1995). In these cases, the consequences of early exposure to toxic substances could occur only after sufficient time has elapsed for the brain-mediated function to appear in a normal developmental sequence. For example, early damage to areas of the brain associated with motor control may not be seen until that time period when infants would be expected to acquire voluntary movement sequences (Lyon and Gaddisseux, 1991).

Finally, there is evidence from both preclinical (Spear et al., 1998) and clinical studies (Mayes et al., 1998) that early exposure to toxic substances may increase the organism's sensitivity or vulnerability to later biological or psychosocial stressors. A similar argument for greater susceptibility has also been made in regard to mechanisms underlying the long-term biomedical consequences of early nutritional deficiencies (Waterland and Garza, 1999). If increased susceptibility to later stresses occurs, fewer developmental-behavioral consequences of early exposure to toxins would appear during the relatively sheltered years of infancy, with increased developmental risk when environmental demands increase as the child gets older. Intraventricular hemorrhage in infancy is one example of increased susceptibility to later stress. The impact of intraventricular hemorrhage on cognitive performance seems to fade through the preschool years, but reappears when the child enters grade school (e.g., a high-demand situation) (Sostek, 1992).

The available evidence supports the importance of a program of systematic and continued monitoring of potential consequences of the addition of new ingredients to infant formulas and systematic in-market follow-up past the infancy period. Such a program is especially important for ingredients with putative or potential biological effects.

Limitations of In-Market Surveillance

Although there is ample justification for a systematic program of in-market monitoring and follow-up surveillance, many factors make such a program difficult to implement, and there are many questions and issues that need to be considered before setting up such a program. There are both logistical problems (e.g., tracking enrolled subjects, continuity of the research team, record retention in follow-up studies, knowing or anticipating what elements to track) and methodological problems (e.g., length of follow-up, confounding factors, reconstructing causality in monitoring studies, having sufficient statistical power to detect what may be subtle, yet clinically significant, adverse effects).

One major issue is the problem of confounding factors. It is obviously difficult, years later, to differentiate effects related to new ingredients from effects due to later exposure to other risk factors. Even with longitudinal studies there are significant logistical problems with associating a single added ingredient in infant formula with any observed outcome. Small differences in the amount or type of a carbohydrate, protein, or fat source may not have the implications of the addition of a bioactive substance completely new to infant formulas.

Throughout life the infant, then the child, and finally the adult, is exposed to a wide variety of food substances with potentially unknown composition. The recent explosion in the use of dietary supplements is an example. Study participants can have difficulty with retrospective recall of prior events, as in the case of whether there was a concurrent use of breastfeeding, even if partial, along with formula feeding. Lifestyle factors may not be obvious, and perhaps not admitted, by the participant. Developmental risk factors not related to infant formulas, such as family stress, also can result in long-term deficits. The longer the follow-up interval, the greater the influence of these possible confounding factors.

The length of time that investigators should study children who were placed on a formula containing a new ingredient presents another set of problems. The length of a study may depend on the nature of the substance added. Formulas and solid infant foods may change over the years of the study so that different cohorts may ingest different nonstudy foods, making comparison difficult.

Defining an adverse event may also be difficult in conducting long-term studies (e.g., beyond 1 year). Investigators must define the duration (permanent or not) and nature of an adverse event. As noted in Chapter 6, there also is a question of what functional domains would need to be assessed when conducting in-market surveillance. Ideally the type of compound added to the formula would dictate what form of follow-up would be most important. However sufficient data may not be available to precisely specify associations between a compound and an affected function.

Finally, as outlined in Chapter 2, sample sizes must be large enough to ensure sufficient statistical power in follow-up studies. As noted in Chapter 6, analysis indicated that the majority of studies investigating the cognitive effects of the addition of LC-PUFAs to infant formulas had insufficient statistical power. The problem of sufficient statistical power to detect adverse consequences is particularly critical when follow-up studies are conducted years after the child has ceased consumption of infant formula. In this situation adverse consequences linked to prior consumption of infant formula may be indirect rather than direct, and thus more subtle, so larger sample sizes will be needed to ensure that there is sufficient statistical power. For example, using a statistical power level of 0.80 as the goal and comparing two types of formula with new ingredients versus a control group (standard formula or breastfed), a sample size of 52 children per group will be required to detect a moderate-effect size difference. In contrast, under the same conditions, a sample size of 140

children per group will be required to detect an intermediate-effect size (i.e., between small and moderate). Unless there are compelling reasons to do otherwise, the committee recommends having sufficient power to detect differences between groups of 0.20 standard deviations or less when estimating sample-size needs in follow-up studies. As is noted later in this chapter, at a population level, even effect sizes of this magnitude can have important clinical implications.

The cost to ensure sufficient power and demonstrate statistical significance of long-term follow-up studies may be a factor to consider when designing such studies. It is beyond the scope of the committee's charge to set cost limits or recommendations.

Current Regulatory Guidelines

As discussed in Chapter 4, formal regulatory guidelines for in-market surveillance do not exist for infant formulas. Section 412 of the Federal Food, Drug and Cosmetic Act states that manufacturers must retain ". . . all complaints and the maintenance of files with respect to, and the review of, complaints concerning infant formulas which may reveal the possible existence of a hazard to health."

As a matter of current practice, infant formula manufacturers routinely conduct passive surveillance via toll-free calls, contact with health care professionals, and reports from their field sales force. Since infants are unable to verbally communicate, any adverse effects must be observed and reported through the parents or caregiver, thus special attention must be paid to detect adverse or unusual reactions when feeding infant formulas containing new ingredients.

Similarly, in Canada there are no explicit guidelines or requirements for in-market surveillance of infant formulas specified under Canada's Food and Drug Regulations in Divisions 16 (Food Additives), 25 (Infant Formula), or 28 (Novel Foods).

OVERVIEW OF RECOMMENDED LEVELS OF ASSESSMENT

RECOMMENDATION: Determine in-market surveillance strategies by implementing a hierarchy of three levels of assessment:

- **Level 1 assessment: passive surveillance.** Toll-free or Internet reporting to formula manufacturers or to the regulatory agency. For example, in August 2002 the U.S. Food and Drug Administration (FDA) established a passive surveillance system for food, cosmetics, and dietary supplements (OSAS, 2002). This system, the Adverse Events Reporting System, will be part of the MedWatch program. Formula manufacturers could add this Internet web site's address to the labeling on each can of formula next to the toll-free reporting number.
- **Level 2 assessment: panel review.** In-market panels to review existing data (both published and proprietary). Issues with regard to the selection and composition of such panels have been discussed extensively in Chapter 4. The same selection and composition recommendations presented earlier also hold with regard to in-market panels.
- **Level 3 assessment: active surveillance.** The following are examples of three different active surveillance options:

  A. Use of follow-up surveillance populations where there is known use of the formula containing the new ingredient or ingredient source (e.g., by using databases

from the Special Supplemental Nutrition Program for Women, Infants and Children or health maintenance organizations).

B. Use of existing pediatric networks as a data source (e.g., Pediatric Research in Office Settings Program of the American Academy of Pediatrics).

C. Clinical follow-up of the original study populations. Such studies would be part of the original study design—not an add-on study.

Based upon testimony to the committee, companies that market infant formulas often rely on level 1 assessment for in-market surveillance. However there are inherent flaws in this assessment level. One such flaw is the risk of underestimating actual negative occurrences given that not all caregivers whose infants experience problems will call in a report. Underestimation is an even greater problem for long-term follow-up since caregivers are not likely to link a child's current problems to intake of infant formula years ago, even when such a linkage may occur. Because the committee does not believe that passive surveillance (level 1 assessment) is sufficient for monitoring all ingredients that might be added to infant formulas, a hierarchy of the options was developed.

Level 1, 2, and 3A assessments would seem most appropriate for in-market monitoring studies, whereas level 2 and 3 assessments would be appropriate for in-market follow-up studies. These levels should not be considered as either-or alternatives. In some cases results from one level could lead to application of a higher level of assessment, as in the case when potential negative effects emerging from in-market panels would suggest that more data be gathered using methods from the higher levels. Figure 7-1 provides an overview of the decision-making process.

In addition to surveillance, a level 2 review of all pertinent data, both published and unpublished, available since the submission to the regulatory agency should be conducted approximately 2 to 4 years after introduction into the market of the product containing the new ingredient.

CRITERIA AND METHODS FOR IN-MARKET SURVEILLANCE

In-Market Monitoring Programs in the First Year

The criteria used to determine the choice of level of assessment and strategies to be utilized are based on the previous discussion in this chapter of conditions under which potential adverse effects of a new ingredient added to infant formula might have been missed in preclinical or clinical trials. Regardless of the level that is chosen, it is expected that systematic data collection procedures (level 1 assessment) or systematic review procedures (level 2 assessment) will ensure that in-market monitoring information will be assessed for each area of function reviewed in Chapter 6.

The choice of level 1 assessment for in-market monitoring is recommended only when *all* of the following conditions occur:

- There is no evidence (presented in the submission) that indicates significant individual or population differences in susceptibility to the ingredient, metabolites, secondary effectors, or source.
- There is no evidence of adverse effects in preclinical or clinical studies, including adverse effects with potentially plausible alternative explanations (e.g., the effects are viewed as the result of random chance or the reviewers believe that there may be methodological or

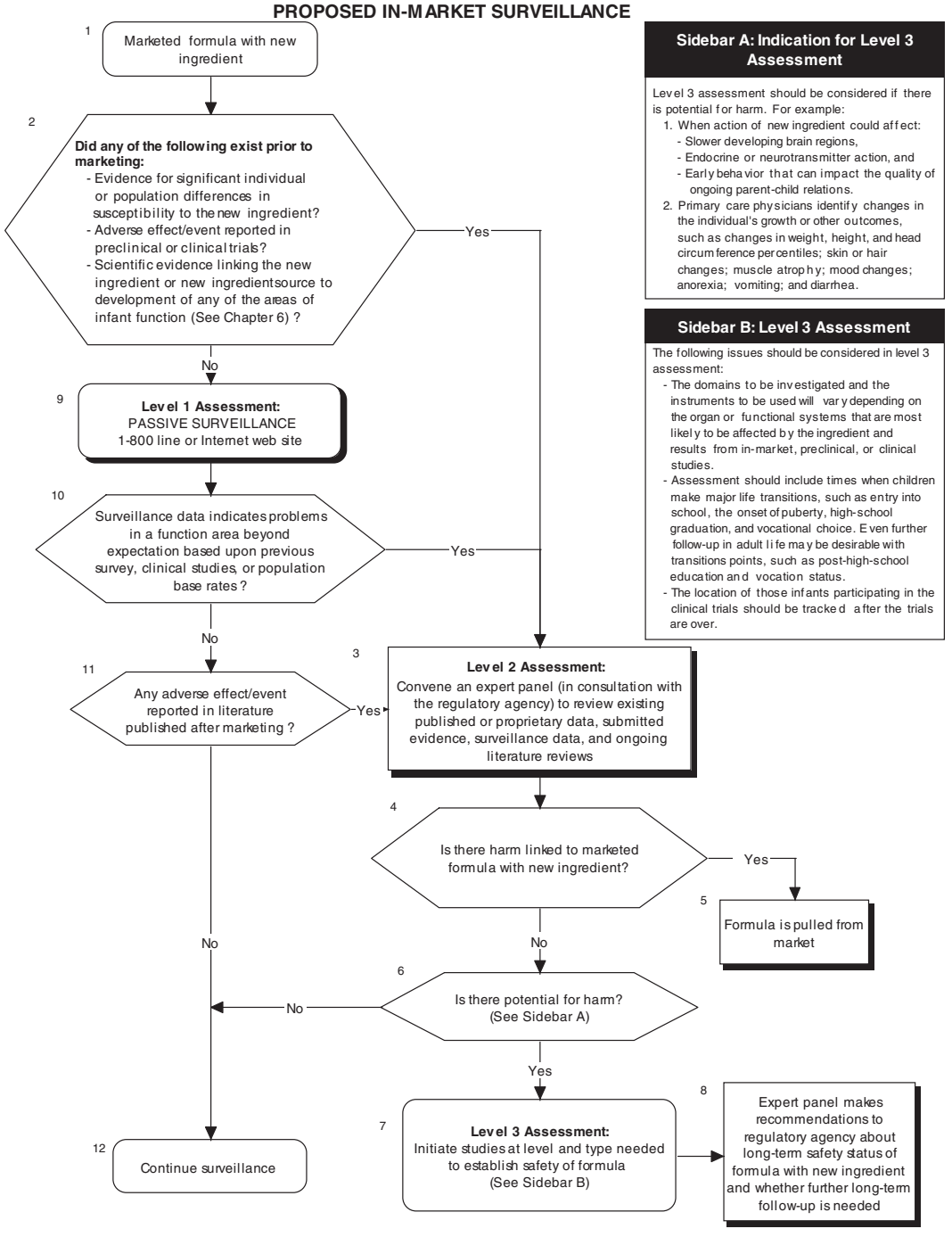

FIGURE 7-1 Proposed in-market surveillance algorithm. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

statistical problems in the studies). This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 1 in-market monitoring would not be warranted since adverse effects were reported.

- A review of the relevant scientific literature indicates that there is no link between the ingredient, metabolites, secondary effectors, or source, and the development of any of the areas of infant function described in Chapter 6.

The choice of level 2 assessment for in-market monitoring must occur when *any one* of the following conditions occurs:

- There is existing evidence for significant individual or population differences in susceptibility to the ingredient, metabolites, secondary effectors, or source.
- There is evidence of some type of adverse effect detected during preclinical or clinical studies. This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 2 in-market monitoring is warranted since adverse effects were reported.
- A review of the relevant scientific literature indicates there is a link between the ingredient, metabolites, secondary effectors, or source and the development of any of the areas of infant function described in Chapter 6.

Level 2 assessments also would be automatically activated if level 1 assessments (in-market toll-free or Internet reporting strategies) are utilized and information indicates problems in a function area over and above what might be expected based upon previous survey or clinical information or population base rates. If the level 2 assessment panel review indicates that existing published or proprietary data show deviations over and above what might be expected based on population base rates, then a study using one or more of the databases in the level 3 assessment would be required. A systematic re-evaluation of safety issues (level 3 assessment studies) is necessary even when individual risk levels are of small effect size, given that even small individual deficits can be magnified when cumulated at a population level (Lester et al., 1998; Rice, 1990; Scott et al., 1994, 1999).

The choice of domains investigated during such a study would depend on the conclusions of the review panel. As noted in Chapter 4, it is essential that each review panel include qualified scientists from all of the domain areas discussed in Chapter 6. If the potential detrimental effects appear to be restricted to a specific organ or functional system, then a detailed investigation of this system with screening assessments of other systems or functions potentially linked to the functioning of the specific organ system would be appropriate. For example, growth could be easily measured during physical examinations of children in surveillance populations by plotting height, weight, head circumference, and perhaps body composition, especially body fat. If the organ or function system involved had known direct links to other organs or functions, then a detailed investigation of the linked systems would be warranted (e.g., given known links between brain and cognitive function, the potential impact of a new ingredient on brain development would require detailed assessment of both brain and cognitive function using the level 3 instruments described in Chapter 6).

In-Market Follow-up Assessments

Potential long-term adverse consequences associated with the addition of ingredients new to infant formulas may not be detected in clinical studies. Therefore, **for every new ingredient to be added to an infant formula, a plan for a systematic approach for in-market**

follow-up of the new formula should be required as part of the submission to the regulatory agency. The studies should specially look for effects at the times when children make major life transitions, such as entry into school. If evidence from the first transition period indicated adverse effects, future studies would be warranted at later transition periods, such as the onset of puberty, high-school graduation, post-high-school education, and vocational choice.

The level of follow-up studies would depend on a number of specific criteria. For example, the level of follow-up surveillance may be influenced by the type of substance added to formulas. For ingredients that change only the flavor, color, or texture, minimal concern may exist for long-term changes, especially if such compounds have a long history of use in food. In contrast, a greater concern may be warranted in the case of nutritional substances. For example, soy-based infant formulas are widely used and account for approximately 40 percent of formula sales in the United States. Most studies of growth and development of infants consuming soy formula follow the infants only to age 1 year. Mendez and coworkers (2002) emphasize the need for follow-up of these infants in the areas of reproduction, immune function, thyroid function, visual acuity, and cognitive function. In addition, evidence indicating that exposure to toxins may increase the organism's responsivity to stress suggests that existing follow-up data should be evaluated or new follow-up data should be collected at time periods when children make life transitions that would increase the level of stress or demand upon the child, such as entry into school.

Based on the evidence cited earlier in this chapter, **higher levels of follow-up assessment levels would be particularly critical when *any one* of the following situations occurs:**

- The action of the new ingredient relates to the development of slower developing brain regions.
- The action of the new ingredient could affect endocrine or neurotransmitter action.
- The action of the new ingredient may have affected early child behavioral changes (e.g., temperament) that can impact upon the quality of ongoing parent-child relations (see Chapter 6).
- Primary-care physicians identify changes in the individual's growth or other outcomes, such as changes in weight, height, and head circumference percentiles; skin or hair changes; muscle atrophy; mood changes; anorexia; vomiting; or diarrhea.
- The action of the new ingredient may have an effect only when individuals are exposed to excess calories or other specific situations.

In the submission to the regulatory agency, the following criteria should be referred to when justifying the strategy proposed for in-market follow-up of new ingredients added to infant formulas.

The choice of level 2 assessment (review panel) for in-market follow-up is recommended when *any one* of the following conditions occurs:

- In-market monitoring reveals adverse effects reported for the new ingredient or ingredient source.
- There is existing evidence for significant individual or population differences in susceptibility to the ingredient, metabolites, secondary effectors, or source.
- There is evidence of adverse effects in preclinical or clinical studies, including adverse effects with potentially plausible alternative explanations (e.g., the effects are viewed as the result of random chance or the reviewers believe that there may be methodological or

statistical problems in the studies). This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 2 in-market monitoring would not be warranted since adverse effects were reported.

- A review of the relevant scientific literature indicates that there is existing evidence linking the new ingredient, metabolites, secondary effectors, or source to the growth and development of organ systems whose functions become apparent after the period of maximum exposure to infant formula, have known long-term direct consequences, or impact upon developmental outcomes that could result in cumulative adverse effects over time.

The choice of level 3 assessments (options A through C) for in-market follow-up must occur when *any one* of the following conditions occurs:

- In-market monitoring reveals a greater than expected number of adverse effects reported for the new ingredient or ingredient source above what might be expected based on population base rates, and an expert panel (level 2 assessment) concludes that there is potential harm for the population. A systematic re-evaluation of long-term safety issues (level 3 assessment studies) is necessary even when individual risk levels are of small effect size, given that even small individual deficits can be magnified when cumulated at a population level (Lester et al., 1998; Rice, 1990; Scott et al., 1994, 1999).
- There is evidence of some type of adverse effect detected during preclinical or clinical trials and an expert panel concludes that there is potential for harm. This means that even if potentially plausible alternative explanations are offered to explain adverse findings, level 2 in-market monitoring is not warranted since adverse effects were reported.
- A review of the relevant scientific literature by an expert panel (level 2 assessment) indicates that new evidence exists linking the ingredient, metabolites, secondary effectors, or source to the growth and development of organ systems whose functions become apparent after the period of maximum exposure to infant formula, have known long-term direct consequences, or a link to developmental outcomes that could result in cumulative adverse effects over time.

The choice between options A through C in level 3 assessment (see earlier section, "Recommended Levels of Assessment") will depend on which populations with known intakes of the formula under question are most available. However the committee recommends as a matter of procedure that efforts be made to keep track of the location of individuals in the original clinical trials (option C), at least through the grade-school years, since this level offers the highest probability of accurately assessing intake of the formula under question. One implication of this recommendation is the importance of ensuring that there is a sufficient sample size in the original clinical trials so that subject attrition does not compromise the level of statistical power in follow-up studies using the original trial population. As discussed earlier in this chapter, sufficient statistical power is especially crucial in follow-up studies since effect sizes may be smaller than in the original clinical trials.

Regardless of which option is chosen, the choice of domains to be investigated in follow-up evaluations and the instruments to be used for each domain will depend on a variety of factors. If the potential detrimental effects appear to be restricted to a specific organ or functional system, then a detailed investigation of this system with screening assessments of other systems or functions potentially linked to the functioning of the specific organ system would be appropriate. For example, anti-infective ingredients (probiotics, prebiotics, lactoferrin) may need follow-up of intestinal function and flora, while immune modulators may

be assessed with both T-cell and B-cell function. As discussed earlier, if the organ or function system involved had known direct links to other organs or functions, then a detailed investigation of the linked systems would be warranted. It also is essential to include qualified scientists from all of the domain areas discussed in Chapter 6 throughout the decision-making process, including the level of follow-up assessment used and the domains where more detailed follow-up will be needed.

Specific instruments chosen must be age appropriate, have documented sensitivity to toxic exposure, and require the least level of invasiveness that still allows sufficient sensitivity. For example, Delaney-Black and colleagues (1998) used routine achievement testing conducted by the child's school system as measures of cognitive functioning in their investigation of the long-term effects upon school-age children who had been exposed to cocaine in the prenatal period. Measures of child behavior were based on parent and teacher responses to standardized rating scales that assessed different dimensions of child adjustment. Follow-up assessments could also look for evidence of referrals to special education, medical services for achievement-related disorders (e.g., attention deficit hyperactivity disorder or learning disabilities), or mental health treatment for associated disorders (e.g., conduct disorder). These sorts of evaluations will need to address confidentiality issues in the evaluation protocol. Again, the decision of which specific measures should be used will require the participation of expert scientists in the domain under consideration (see Chapter 6).

Existing Models for In-Market Surveillance

Despite the difficulties inherent in drawing useful conclusions from long-term, in-market surveillance studies, possible research models for such studies exist. For example, an opportunity to study long-term effects of nutritional changes may be possible with the beginning of the National Children's Study, authorized by Congress in 2000. This study proposes to follow 100,000 children from birth to age 21, with a specific focus on environmental influences on growth and development (National Children's Study, 2003). Nutrition will obviously be a major focus of this study. Data from this type of longitudinal follow-up study might be used to determine if there were increases in specific adverse events, over what would be expected at a population level, after a formula containing a new ingredient was introduced to the general marketplace.

In terms of actual research models, a recent analysis of the outcomes of infants placed on soy formula is an example of extending what was originally a short-term study of growth of infants between the age of 9 days and 16 weeks (Strom et al., 2001). The original study was performed from 1965 to 1978. The investigators returned to their original population to investigate possible long-term endocrinological and reproductive outcomes of infants who had received soy formula containing phytoestrogens. Of the 952 subjects in the original cohort, the investigators had to reject 48 subjects because of exposure to formulas other than soy. Of the remaining 904 subjects, 51 were lost to follow-up and 42 refused to participate. Overall, 90 percent of the original cohort was located and data were available from 85 percent. This study was unable to identify any effect on endocrinological or general health. While this type of study is difficult to conduct because both patients and investigators move, it shows that formula follow-up studies are possible when there is tenacity and continuity of investigators.

Other research models also exist. An efficacy and safety study of an infant formula with added LC-PUFAs was carried out for 18 months (Lucas et al., 1999). Another study of the addition of LC-PUFAs with a follow-up period of 14 months (Auestad et al., 2001) was extended, with the original study group of patients, to a follow-up period of 39 months

(Auestad et al., 2003). In 1978 and 1979, two soy-based formulas were released that contained markedly decreased chloride content. The result was a large number of cases of what was termed the "chloride deficiency syndrome" diagnosed between 1 and 6 months of age (Roy, 1984). These infants presented with failure to thrive, lethargy, muscular weakness, and loss of appetite. Laboratory analysis revealed metabolic alkalosis, hypochloremia, hypokalemia, and hyponatremia. Nine- and 10-year follow-up of some of these infants showed no measurable deficits in cognitive development (Willoughby et al., 1990).

The Strom and colleagues (2001) soy study, as well as other longitudinal studies, such as the Framingham Heart Study (NHLBI, 2002) and the Harvard Physicians Health Study (Steering Committee of the Physicians' Health Study Research Group, 1989), demonstrates that drawing valid conclusions from long-term surveillance studies can be accomplished, even considering the multiple methodological problems inherent in such studies.

SUMMARY

The two components of in-market surveillance include monitoring for adverse effects in infants after a formula has been introduced and long-term follow-up to ensure that there are no delayed effects. There are a variety of logistical and methodological problems associated with in-market surveillance, especially in regard to long-term follow-up (e.g., tracking subjects, reconstructing causality). However there is a crucial need for such long-term surveillance (e.g., brain areas that are adversely affected by new ingredients may not functionally become apparent until later in development, or early exposure to a toxin may increase susceptibility to later exposure to toxins). The committee recommends a systematic plan for both continued in-market monitoring and long-term surveillance as an essential part of each safety evaluation seeking to add new ingredients to infant formulas. The committee provides three possible levels of assessment for in-market surveillance, along with criteria to decide which surveillance level is appropriate for a new ingredient.

REFERENCES

Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, Singer LT, Montalto MB, Jacobs JR, Qiu W, Bornstein MH. 2001. Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics* 108:372–381.

Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, Halter R, Qiu W, Jacobs JR, Connor WE, Connor SL, Taylor JA, Neuringer M, Fitzgerald DM, Hall RT. 2003. Visual, cognitive, and language assessments at 39 months: A follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* 112:e177–e183.

Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. 1993. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 341:938–941.

Beaton GH. 1986. 1986 EV McCollum International Lectureship in Nutrition. Toward harmonization of dietary, biochemical, and clinical assessments: The meanings of nutritional status and requirements. *Nutr Rev* 44:349–358.

Bellinger DC. 1995. Interpreting the literature on lead and child development: The neglected role of the "experimental system." *Neurotoxicol Teratol* 17:201–212.

Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL, Waternaux C. 1991. Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics* 87:219–227.

Bendersky M, Alessandri SM, Lewis M. 1996. Emotions in cocaine-exposed infants. In: Lewis M, Sullivan MW, eds. *Emotional Development in Atypical Children.* Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 89–108.

Bengtsson T, Lindstrom M. 2000. Childhood misery and disease in later life: The effects on mortality in old age of hazards experienced in early life, southern Sweden, 1760–1894. *Popul Stud (Camb)* 54:263–277.

Chasnoff IJ, Burns KA, Burns WJ. 1987. Cocaine use in pregnancy: Perinatal morbidity and mortality. *Neurotoxicol Teratol* 9:291–293.

Clarke ADB, Clarke AM. 1981. "Sleeper effects" in development: Fact or artifact? *Dev Rev* 1:344–360.

Delaney-Black V, Covington C, Templin T, Ager J, Martier S, Compton S, Sokol R. 1998. Prenatal coke: What's behind the smoke? *Ann N Y Acad Sci* 846:277–288.

Fried PA. 1989. Cigarettes and marijuana: Are there measurable long-term neurobehavioral teratogenic effects? *Neurotoxicology* 10:577–584.

Galler JR, Tonkiss J. 1998. The effects of prenatal protein malnutrition and cocaine on the development of the rat. *Ann N Y Acad Sci* 846:29–39.

Griffith DR, Azuma SD, Chasnoff IJ. 1994. Three-year outcome of children exposed prenatally to drugs. *J Am Acad Child Adolesc Psychiatry* 33:20–28.

Jackson AA. 2000. Nutrients, growth, and the development of programmed metabolic function. *Adv Exp Med Biol* 478:41–55.

Jacobson SW, Jacobson JL. 2000. Teratogenic insult and neurobehavioral function in infancy and childhood. In: Nelson CA, ed. *The Minnesota Symposia on Child Psychology. Vol 31: The Effects of Early Adversity on Neurobehavioral Development*. Mahwah, NJ: Lawrence Erlbaum Associates. Pp. 61–112.

Leech SL, Richardson GA, Goldschmidt L, Day NL. 1999. Prenatal substance exposure: Effects on attention and impulsivity of 6-year-olds. *Neurotoxicol Teratol* 21:109–118.

Lester BM, LaGasse LL, Seifer R. 1998. Cocaine exposure and children: The meaning of subtle effects. *Science* 282:633–634.

Leviton A, Bellinger D, Allred EN, Rabinowitz M, Needleman H, Schoenbaum S. 1993. Pre- and postnatal low-level lead exposure and children's dysfunction in school. *Environ Res* 60:30–43.

Lucas A, Stafford M, Morley R, Abbott R, Stephenson T, MacFadyen U, Elias-Jones A, Clements H. 1999. Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: A randomised trial. *Lancet* 354:1948–1954.

Lyon G, Gadisseux J-F. 1991. Structural abnormalities of the brain in developmental disorders. In: Rutter M, Casaer P, eds. *Biological Risk Factors for Psychosocial Disorders*. New York: Cambridge University Press. Pp. 1–19.

Mayes LC, Grillon C, Granger R, Schottenfield R. 1998. Regulation of arousal and attention in preschool children exposed to cocaine prenatally. *Ann N Y Acad Sci* 846:126–143.

Mendez MA, Anthony MS, Arab L. 2002. Soy-based formula and infant growth and development: A review. *J Nutr* 132:2127–2130.

National Children's Study. 2003. *What is the National Children's Study?* Available at http://nationalchildrensstudy.gov/about/overview.cfm. Accessed November 21, 2003.

NHLBI (National Heart, Lung, and Blood Institute). 2002. *Framingham Heart Study. 50 Years of Research Success*. Online. Available at http://www.nhlbi.nih.gov/about/framingham/index.html. Accessed July 1, 2002.

O'Connor MJ, Sigman M, Kasari C. 1993. Interactional model for the association among maternal alcohol use, mother-infant interaction, and infant cognitive development. *Inf Behav Dev* 16:177–192.

OSAS (Office of Scientific Analysis and Support). 2002. *Letter to Stakeholders. Announcing CAERS—The CFSAN Adverse Event Reporting System*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~dms/caersltr.html. Accessed January 21, 2003.

Rice DC. 1990. The health effects of environmental lead exposure: Closing Pandora's box. In: Russell RW, Flattau PE, Pope AM, eds. *Behavioral Measures of Neurotoxicity: Report of a Symposium*. Washington, DC: National Academy Press. Pp. 243–267.

Richardson GA. 1998. Prenatal cocaine exposure. A longitudinal study of development. *Ann N Y Acad Sci* 846:144–152.

Richardson GA, Conroy ML, Day NL. 1996. Prenatal cocaine exposure: Effects on the development of school-age children. *Neurotoxicol Teratol* 18:627–634.

Romano AG, Harvey JA. 1998. Prenatal cocaine exposure: Long-term deficits in learning and motor performance. *Ann N Y Acad Sci* 846:89–108.

Roy S III. 1984. The chloride depletion syndrome. *Adv Pediatr* 31:235–257.

Ruff HA. 1999. Population-based data and the development of individual children: The case of low to moderate lead levels and intelligence. *J Dev Behav Pediatr* 20:42–49.

Russell RW. 1990. Neurobehavioral time bombs: Their nature and their mechanisms. In: Russell RW, Flattau PE, Pope AM, eds. *Behavioral Measures of Neurotoxicity: Report of a Symposium*. Washington, DC: National Academy Press. Pp. 206–225.

Rutter M, Pickles A. 1991. Person-environment interactions: Concepts, mechanisms, and implications for data analysis. In: Wachs TD, Plomin R, eds. *Conceptualization and Measurement of Organism Environment Interaction.* Washington, DC: American Psychological Association. Pp. 105–141.

Schneider ML, Roughton EC, Koehler AJ, Lubach GR. 1999. Growth and development following prenatal stress exposure in primates: An examination of ontogenetic vulnerability. *Child Dev* 70:263–274.

Scott KG, Shaw KH, Urbano JC. 1994. Developmental epidemiology. In: Friedman SL, Haywood HC, eds. *Developmental Follow-up. Concepts, Domains, and Methods.* San Diego: Academic Press. Pp. 351–374.

Scott KG, Avchen RN, Hollomon HA. 1999. Epidemiology of child developmental problems: The extent of the problems of poor development in children from deprived backgrounds. *Food Nutr Bull* 20:34–44.

Singer LT, Arendt R, Minnes S, Farkas K, Salvator A, Kirchner HL, Kliegman R. 2002. Cognitive and motor outcomes of cocaine-exposed infants. *J Am Med Assoc* 287:1952–1960.

Sostek AM. 1992. Prematurity as well as intraventricular hemorrhage influence development outcome at 5 years. In: Friedman SL, Sigman MD, Sigel IE, eds. *The Psychological Development of Low-Birthweight Children. Annual Advances in Applied Developmental Psychology.* Vol 6. Norwood, NJ: Ablex Publishing. Pp. 259–274.

Spear LP, Campbell J, Snyder K, Silveri M, Katovic N. 1998. Animal behavior models. Increased sensitivity to stressors and other environmental experiences after prenatal cocaine exposure. *Ann N Y Acad Sci* 846:76–88.

Spencer PS. 1990. Chemical time bombs: Environmental causes of neurodegenerative diseases. In: Russell RW, Flattau PE, Pope AM, eds. *Behavioral Measures of Neurotoxicity: Report of a Symposium.* Washington, DC: National Academy Press. Pp. 268–284.

Steering Committee of the Physicians' Health Study Research Group. 1989. Final report on the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med* 321:129–135.

Strom BL, Schinnar R, Ziegler EE, Barnhart KT, Sammel MD, Macones GA, Stallings VA, Drulis JM, Nelson SE, Hanson SA. 2001. Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. *J Am Med Assoc* 286:807–814.

Wachs TD. 2000. *Necessary but Not Sufficient. The Respective Roles of Single and Multiple Influences on Individual Development.* Washington, DC: American Psychological Association.

Wasserman GA, Liu X, Popovac D, Factor-Litvak P, Kline J, Waternaux C, LoIacono N, Graziano JH. 2000. The Yugoslavia Prospective Lead Study: Contributions of prenatal and postnatal lead exposure to early intelligence. *Neurotoxicol Teratol* 22:811–818.

Waterland RA, Garza C. 1999. Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 69:179–197.

Weiss B. 1995. Incipient hazards of cocaine: Lessons from environmental toxicology. In: Lewis M, Bendersky M, eds. *Mothers, Babies, and Cocaine: The Role of Toxins in Development.* Hillsdale, NJ: Lawrence Erlbaum Associates. Pp. 41–55.

Willoughby A, Graubard BI, Hocker A, Storr C, Vietze P, Thackaberry JM, Gerry MA, McCarthy M, Gist NF, Magenheim M, Berendes H, Rhoads GG. 1990. Population-based study of the developmental outcome of children exposed to chloride-deficient infant formula. *Pediatrics* 85:485–490.

Winneke G. 1990. Neurobehavioral toxicity of selected environmental chemicals: Clinical and subclinical aspects. In: Russell RW, Flattau PE, Pope AM, eds. *Behavioral Measures of Neurotoxicity: Report of a Symposium.* Washington, DC: National Academy Press. Pp. 226–242.

A

Acronyms and Glossary

AAP	American Academy of Pediatrics
ADME	Absorption, distribution, metabolism, and excretion
ALAT	Alanine amino transferase
Algorithm	"A step-by-step procedure for solving a problem that contains conditional logic (if/then) statements" (SMDMC, 1992)
ARA	Arachidonic acid
ASAT	Aspartate amino transferase
CDC	U.S. Centers for Disease Control and Prevention
CFSAN	U.S. Center for Food Safety and Applied Nutrition
CNS	Central nervous system
DEXA	Dual X-ray absorptiometry
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intakes
Efficacy	The capacity to produce an intended effect under the realistic situation of product use
FDA	U.S. Food and Drug Administration
FDA Redbook	*Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft* (OFAS, 2001) and *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients* (OFAS, 2003)
FD&C Act	Federal Food, Drug and Cosmetic Act

Food additive	"Any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food . . . , if such substance is not generally recognized, among experts qualified by the scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case as a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use" (FD&C Act, Section 201(s))
GFR	Glomerular filtration rate
GMP	Good Manufacturing Practices
GRAS	Generally Recognized as Safe
Harm	The nature of the undesired outcome (usually a health outcome) associated with a hazard; often expressed in terms such as "cost"
Hazard	Some substance or combination of substances (organic or inorganic, or in some cases psychological) that may, under some circumstances and/or for some individuals, produce undesired health-related outcomes.
HDL	High-density lipoprotein
HHS	U.S. Department of Health and Human Services
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
IgA, IgE, IgG, IgM	Immunoglobulin A, E, G, M
Infant formula	A food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk (FD&C Act, Section 201(z))
IOM	Institute of Medicine
IVH	Intraventricular hemorrhage
LC-MS	Liquid chromatography-mass spectrometry
LC-PUFAs	Long-chain polyunsaturated fatty acids
LD_{50}	Lethal dose for 50 percent of the animals used in a study
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
LSRO	Life Sciences Research Office
MRI	Magnetic resonance imaging
NHST	Null hypothesis significance test
Novel food	"(a) a substance, including a microorganism, that does not have a history of safe use as a food; (b) a food that has been manufactured, prepared, preserved or packaged by a process that (i) has not been previously applied to that food, and (ii) causes the food to

	undergo a major change; and (c) a food that is derived from a plant, animal or microorganism that has been genetically modified such that (i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism, (ii) the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism, or (iii) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism" (Canada, 2001)
Prebiotic	"a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995)
Probiotic	"a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989)
Quality factors	Factors necessary to demonstrate that the infant formula, as prepared for market, provides nutrients in a form that is bioavailable and safe as shown by evidence that demonstrates that the formula supports the healthy growth when fed as the sole source of nutrition (FDA, 1996)
Risk	Likelihood that an adverse event will occur given exposure to a hazard
Safety	A reasonable certainty of no harm or, in some systems, a reasonable balance between costs (harm) and benefits
Term infants	Infants delivered between gestational age of 37 to 42 weeks with birth weights of 2.5 kg or more; safety parameters and committee reviews were limited to healthy term infants with no chronic disease
TLC	Thin layer chromatography
Type I error	The probability of falsely rejecting the null hypothesis (i.e., the chance of concluding that a systematic relationship exists in the population when it does not)
Type II error	Failure to detect a real effect; occurs when one fails to reject a false null hypothesis
UL	Tolerable Upper Intake Level
VLDL	Very low-density lipoprotein
WHO	World Health Organization

REFERENCES

Canada. 2001. *Departmental Consolidation of the Food and Drugs Act and the Food and Drug Regulations. (Food and Drugs Act)*. Ottawa: Minister of Public Works and Government Services Canada.

FDA (Food and Drug Administration). 1996. Current good manufacturing practice, quality control procedures, quality factors, notification requirements, and records and reports, for the production of infant formula. Proposed rule. *Fed Regist* 61:36153–36219.

Fuller R. 1989. Probiotics in man and animals. *J Appl Bacteriol* 66:365–378.

Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota. Introducing the concept of prebiotics. *J Nutr* 125:1401–1412.

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS. 2003. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

SMDMC (Society for Medical Decision Making Committee). 1992. Proposal for clinical algorithm standards. Society for Medical Decision Making Committee on Standardization of Clinical Algorithms. *Med Decis Making* 12:149–154.

B

Composition of Infant Formulas and Human Milk for Feeding Term Infants in the United States

TABLE B-1 Composition of Selected Formulas Marketed for Feeding to Term Infants in the United States

Component	Milk-Based		
	Lactose Free	Whey Predominant	Contain DHA and ARA[a]
Protein equivalent (g)	14	14–16	14
Fat (g)	31–37	36–37	36–37
Fatty acids			
DHA (%)	NA[b]	NA	0.15
ARA (%)	NA	NA	0.40
Polyunsaturated (%)	—	22	—
Saturated (%)	—	45	—
Monounsaturated (%)	—	33–38	—
Linoleic (mg)	8,784	3,360–6,205	5,810–6,757
Carbohydrate (g)	72–74	71–81	73–74
Minerals			
Calcium (mg)	550–568	423–527	527
Phosphorus (mg)	370–378	263–358	284–358
Magnesium (mg)	41–540	47–54	41–54
Iron (mg)	12	11–12	12
Zinc (mg)	5–7	4–7	5–7
Manganese (µg)	34–101	51–101	34–101
Copper (µg)	510–608	470–584	527–608
Iodine (µg)	61–101	11–68	41–68
Selenium (µg)	15–19	14–19	12–19
Sodium (mg)	200–203	148–182	162–182
Potassium (mg)	723–740	558–730	709–730
Chloride (mg)	439–450	376–431	426–439
Vitamins			
Vitamin A (IU)	2,000–2027	2,016–2,190	2,027
Vitamin D (IU)	405–410	403–438	228–405
Vitamin E (IU)	14–20	9–15	10–13
Vitamin K (µg)	54	54–60	54
Thiamin (µg)	540–676	438–672	541–676
Riboflavin (µg)	950–1,014	946–1,008	946–1,014
Niacin (µg)	6,800–7,095	5,475–6,757	6,757–7,095
Vitamin B_6 (µg)	405–410	405–548	405
Folic Acid (µg)	101–108	50–110	101–108
Vitamin B_{12} (µg)	2	1.3–2	2
Pantothenic acid (µg)	3,041–3,400	2,117–3,378	3,040–3,378
Biotin (µg)	20–30	15–35	20–30
Vitamin C (mg)	61–81	57–81	61–81
Other nutrients			
Choline (mg)	81–108	81–101	81–108
Inositol (mg)	29–115	28–131	32–41
Nucleotides (mg)	—	28–34	—
Potential renal solute load (mOsm/L)	132–180	99–132	270

NOTE: Nutrient unit/L, unless otherwise noted.
[a]DHA = docosahexaenoic acid, ARA = arachidonic acid.
[b]NA = not applicable.
[c] — = not available.
SOURCE: Melanie Fairchild-Dzanis, personal communication, Nestle (August 26, 2002); IOM (1997, 1998, 2000, 2001, 2002, 2004); Mead Johnson Nutritionals (1999); Ross Products Division (2001), Anna Skulimowski, personal communication, Wyeth Nutrition (August 26, 2002, and December 11, 2002).

	Human Milk		Adequate Intake (per day)	
Isolated Soy-Protein Based	0–6 Months	7–12 Months	0–6 Months	7–12 Months
17–20	11.7	12.1	9.1	9.9
33–37	40	40	31	31
NA	1.58	1.58	0.5 g	0.5 g
NA	—[c]	—	—	—
24	15.6	15.6	4.9 g	5.1 g
43	—	—	—	—
33	—	—	—	—
3,360–6,757	560	560	440	460
68–74	74	74	60	95
605–710	264	210	210	270
409–560	124	124	100	275
50–74	34	34	30	75
12	0.35	0.35	0.27	—
4–8	2.5	0.85	2.0	—
169–228	3.5	3.5	3	600
470–804	250	200	200	220
60–101	146	146	110	130
14–19	18	18	15	20
202–297	160	130	120	370
706–810	500	500	400	700
376–540	—	—	180	570
2,000–2,077	1,616	1,616	1,333	1,666
402–410	15.9 ± 8.6	15.9 ± 8.6	200	200
9–20	7.3	7.3	6	7.5
52–74	250	250	2	2.5
402–672	21 ± 0.04	21 ± 0.04	20	30
608–1,008	35	35	30	40
5,040–9,122	180	180	200	400
402–420	13	13	10	30
50–108	65	65	65	80
2–3	0.4	0.4	0.4	0.5
3,024–5,068	220	220	170	180
20–52	6	6	5	6
56–107	50	50	40	50
54–87	160	160	125	150
28–121	—	—	—	—
—	—	—	—	—
130–180	—	—	—	—

REFERENCES

IOM (Institute of Medicine). 1997. *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press.

IOM. 1998. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press.

IOM. 2000. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Washington, DC: National Academy Press.

IOM. 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academy Press.

IOM. 2002. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: National Academy Press.

IOM. 2004. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*. Washington, DC: The National Academies Press.

Mead Johnson Nutritionals. 1999. *Pediatric Products Handbook*. Evansville, IN: Mead Johnson.

Ross Products Division. 2001. *Pediatric Nutritionals Product Guide*. Columbus, OH: Ross Products Division/ Abbott Laboratories.

C

Redbook Table of Contents

d. Improving the Risk Assessment Process/Safety Assessment
5. References

C. Immunotoxicity Studies
1. Immunity: A Brief Review
2. Key Concepts in Immunotoxicity Testing
3. Indicators of Possible Immune Toxicity
4. Expanded Type 1 Immunotoxicity Tests
5. Type 2 Immunotoxicity Tests
6. Relevance of Primary Indicators of Immune Toxicity to Health
7. Adequacy and Reliability of Primary Indicators of Immune Toxicity
8. Recommendations for Further Immunotoxicity Testing when Primary Indicators are Positive
9. Animal Models for Immunotoxicity Tests
10. Recommended Strategy for Assessing the Immunotoxic Potential of Food Ingredients
11. Conclusion

Chapter VI. Human Studies
A. Clinical Evaluation of Food Ingredients
1. General Considerations for Clinical Studies of Food Ingredients
2. Specific Considerations for Clinical Studies of Food Ingredients
3. Sequence of Clinical Studies for Food Ingredients
4. Submitting Reports of Clinical Studies on Food Ingredients
5. Appendix A — Principles of Institutional Review and Informed Consent

B. Epidemiology Studies

Chapter VII. Emerging Issues
A. Introduction
B. Macro-Additives
C. Safety of Food Ingredients Developed by Biotechnology
D. Enzymes
E. Microbially Derived Food Ingredients
F. Advances in the Development of Alternatives to Toxicity Testing
G. Heritable and Somatic Genetic Toxicity

Chapter VIII. Glossary: Acronyms and Definitions

NOTE: Food ingredients include: direct food additives, color additives used in food, Generally Recognized as Safe substances, food contact substances, and constituents or impurities of any of the above. **Bolded** text denotes a section that has been finalized and is found only online in the Redbook 2000 (OFAS, 2003); the other information is found only in Redbook II-Draft (OFAS, 2001).

REFERENCES

OFAS (Office of Food Additive Safety). 2001. *Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food. Redbook II-Draft*. Washington, DC: OFAS, Center for Food Safety and Applied Nutrition, Food and Drug Administration.

OFAS. 2003. *Redbook 2000. Toxicological Principles for the Safety of Food Ingredients*. Online. Center for Food Safety and Applied Nutrition, Food and Drug Administration. Available at http://www.cfsan.fda.gov/~redbook/red-toca.html. Accessed November 19, 2003.

D

Applying the Recommended Approaches

The special needs and vulnerabilities of infants require a clear and complete set of guidelines to assess the safety of infant formulas. Guidelines must also provide an appropriate level of flexibility to address the multitude and diversity of possible new ingredients. It is not realistic or desirable to provide specific recommendations for each potential new ingredient. Thus the committee recommends that the manufacturer or notifier and an expert review panel establish the relative importance of potential adverse effects for each new ingredient and determine the types of preclinical and clinical studies and in-market surveillance needed to accurately assess safety.

The committee was requested to apply the recommended tools and approaches to the specific situation of adding long-chain polyunsaturated fatty acids (LC-PUFAs) to infant formulas and to consider another example of a specific ingredient, if appropriate. Probiotics was selected as the second case study to demonstrate the flexibility of the algorithms when analyzing a "complex" ingredient comprised of a variety of different components that would be contained in a microorganism or microorganism mix. This appendix describes the steps in the committee's recommended approaches that should be considered when assessing the safety of LC-PUFAs and probiotics.

As discussed in Chapter 1, algorithms diagram the safety assessment process into a step-by-step decision tree. The algorithms presented in Chapters 4 through 7 are provided to summarize the appropriate level of assessment by considering the harm and the potential adverse effects of a new ingredient. The approaches presented in this appendix are not meant to provide all of the information, events, or tests that need to be assessed to ensure safety, but rather to exemplify needed steps in the review process. The corresponding chapters provide more information on specific levels and tests.

The committee emphasizes that the purpose of these case studies is not to make conclusions about the completeness of the information or the safety of the new ingredient, but to point out assessment processes or steps that need to be considered. The algorithms that follow utilize an asterisk (*) with corresponding text underlined to indicate steps that the committee concluded could have been included if the notifier had used the committee's

recommended algorithms to guide decisions about the type of safety assessments to apply. The asterisk with corresponding text underlined does not imply that the step was not performed or considered by the qualified experts, but that the information was not available for review by the committee. Note that under the proposed recommendation (Chapter 4), the expert panel can choose to include or not include certain tests in the submission.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS

For the case of LC-PUFAs, the committee applied the information given to the Food and Drug Administration available through the Freedom of Information Act in Generally Recognized as Safe (GRAS) Notices 000041 and 000080 to its recommended algorithms, comparing the steps taken by the manufacturer, notifier, or expert panel to determine the safety of ARASCO (arachidonic acid-rich single-cell oil) and DHASCO (docosahexaenoic acid-rich single-cell oil) in infant formulas. Figure D-1 provides an overview of the proposed process. The sources of ARASCO and DHASCO have no prior use in foods in the United States, but they are used in infant formulas in Europe.

Preclinical Studies

As shown in Figure D-2, structure, stability, and solubility characterization studies are an important part of preclinical assessment. Figure D-3 illustrates that level 2 assessments would have been applied in determining the safety of the LC-PUFAs. These in-depth measures of the organ and neurological systems would further investigate abnormalities and/or are theoretically related to structure or function. In this case it appeared that there were some minor effects on organ systems. It is not obvious which of the proposed testing regimes in Chapter 5 were followed. The nonhuman primate studies were limited. These are considered an appropriate model to study changes in general behavior and speed of neural processing in response to the addition of LC-PUFAs. Chapter 5 provides more details on structure, stability, and solubility characterization, as well as the committee's recommendations for level 2 assessments.

Clinical Studies

As seen in the overview of the proposed clinical guidelines (Figures D-4, D-5, and D-6), it is not clear whether assessments of body composition, immune response, auditory function, and temperament were conducted. Several of these tests (to be determined by expert panels), applied at level 2 or level 3, are especially important to determine the safety of LC-PUFAs because theoretical safety concerns exist. For example, LC-PUFAs affect immune response, and they have been linked to neural development. Chapter 6 provides the committee's findings and recommendations on body composition and immune, auditory, and temperament assessment.

In-Market Surveillance

Figure D-7 illustrates the levels of proposed in-market surveillance. Selection of an appropriate type of in-market surveillance should be based on theoretical concerns about the new ingredient and/or results from preclinical and clinical studies. As long as preclinical and clinical studies are properly conducted, adverse outcomes should be rare and it would take a considerable period of time to collect sufficient data in order to reaffirm the GRAS status

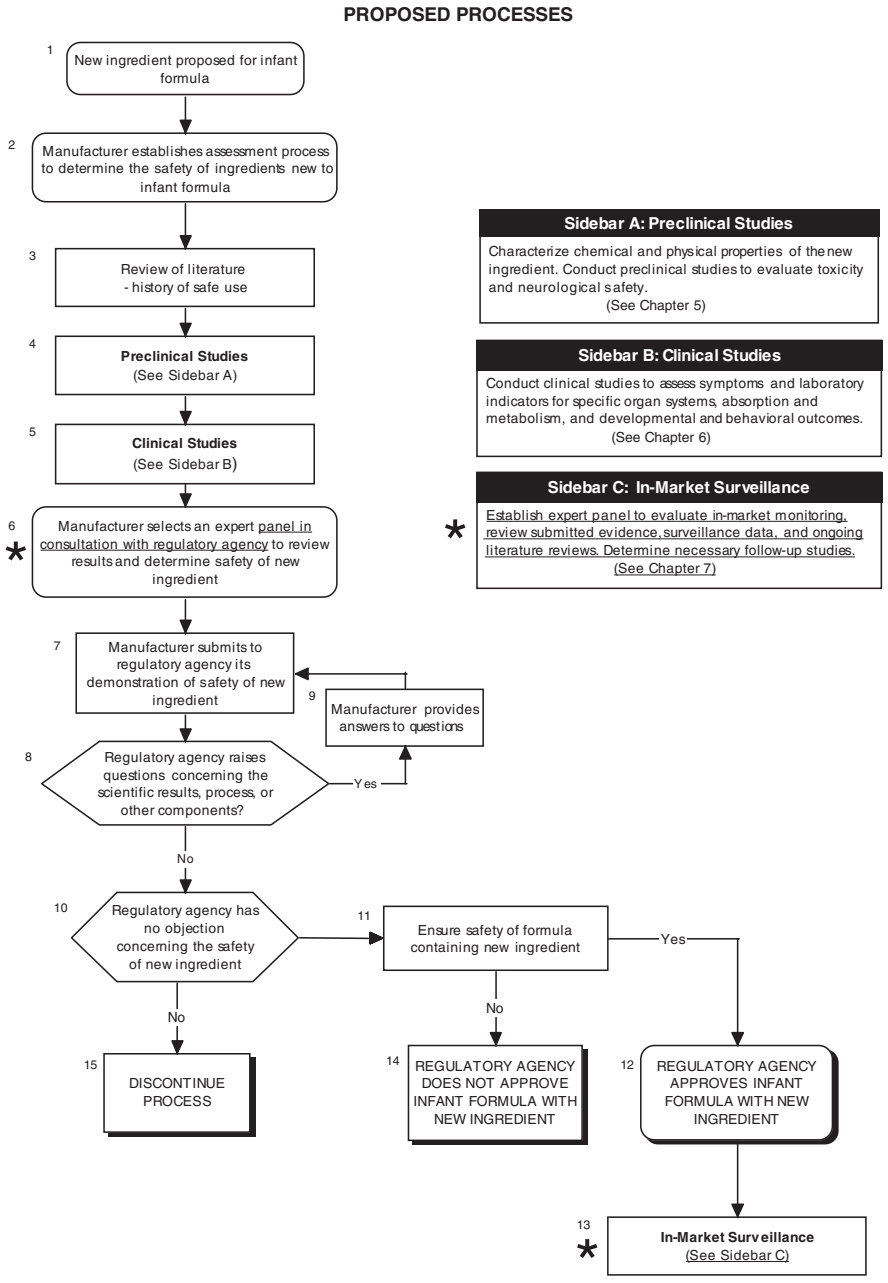

FIGURE D-1 Proposed process for evaluating the safety of ingredients new to infant formulas algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. In-market assessment should be planned in conjunction with preclinical and clinical testing. This algorithm is modeled after the U.S. Generally Recognized as Safe Notification process; similar schemes can be adapted to other regulatory processes. [ ] = a state or condition, < > = a decision point, [ ] = an action, sidebar = an elaboration of recommendation or statement.

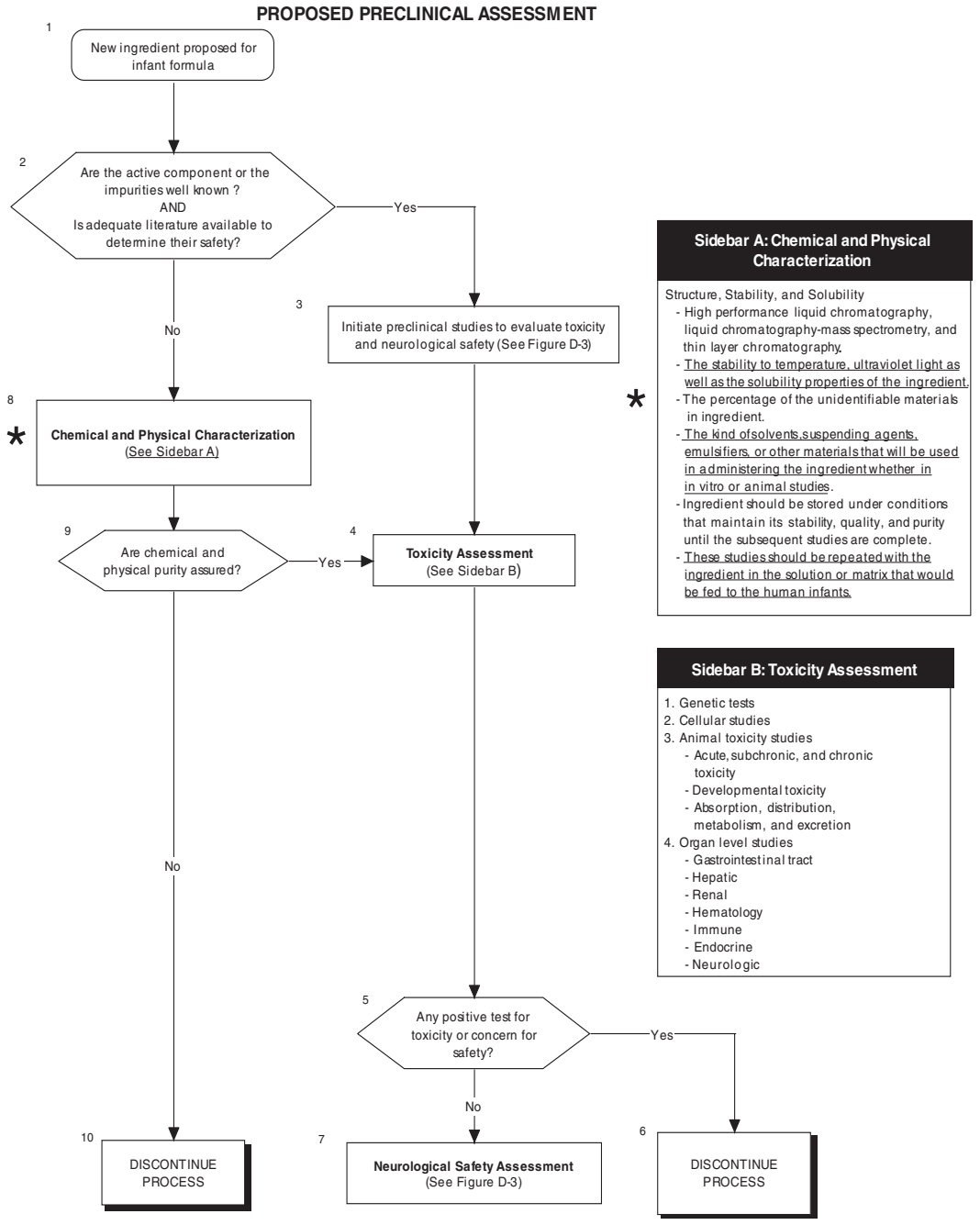

FIGURE D-2 Proposed preclinical assessment algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

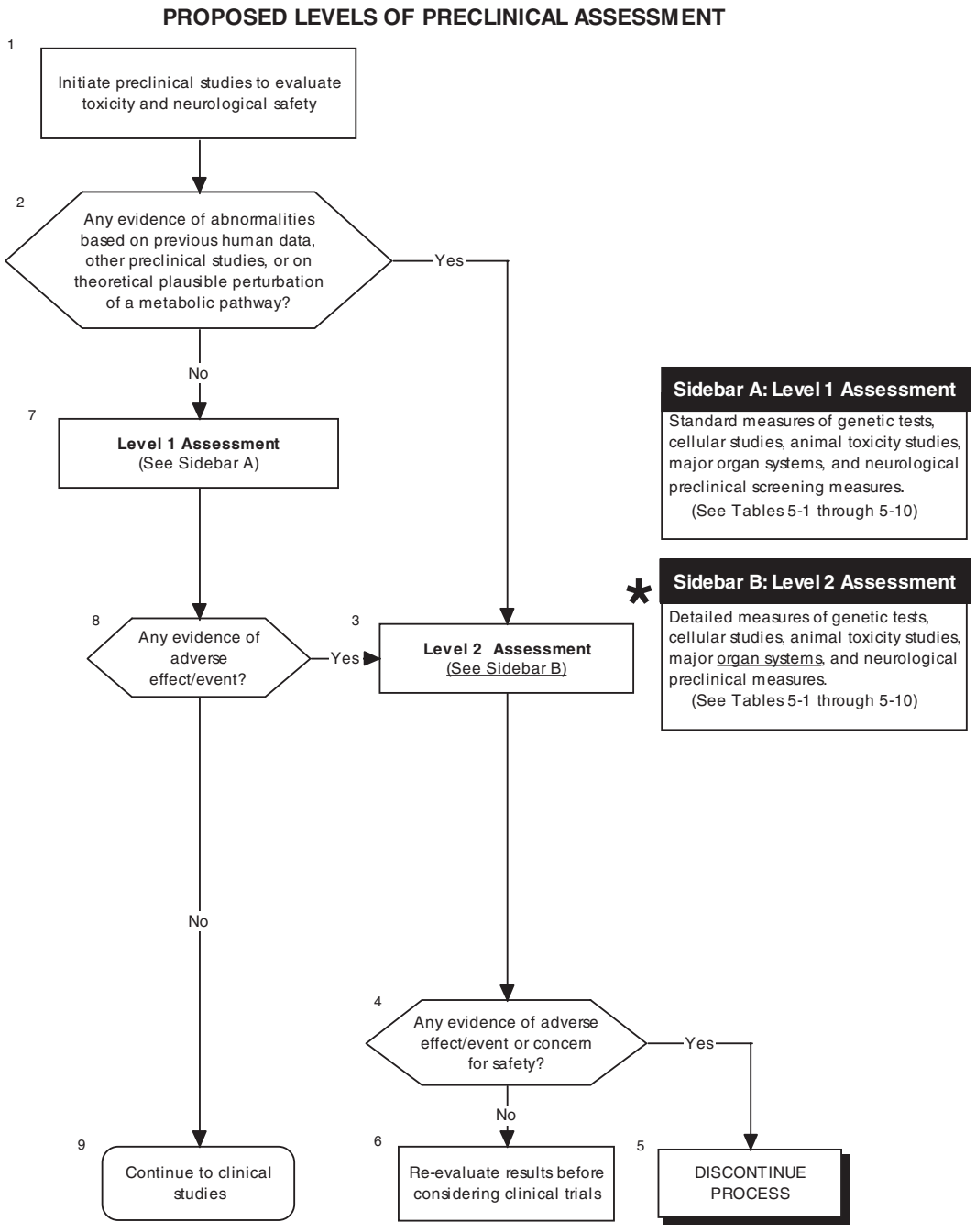

FIGURE D-3 Proposed levels of preclinical assessment algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

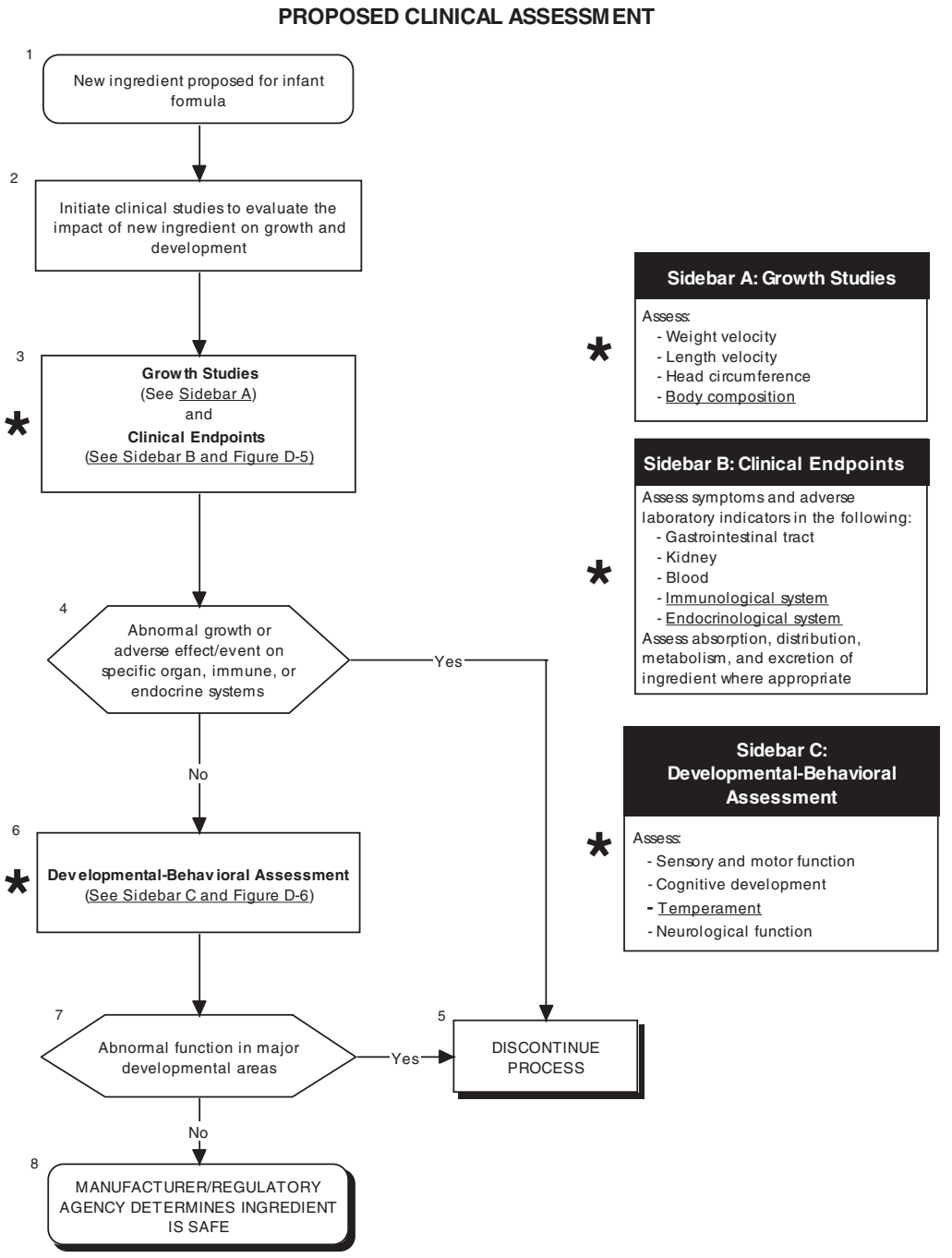

FIGURE D-4 Proposed clinical assessment algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

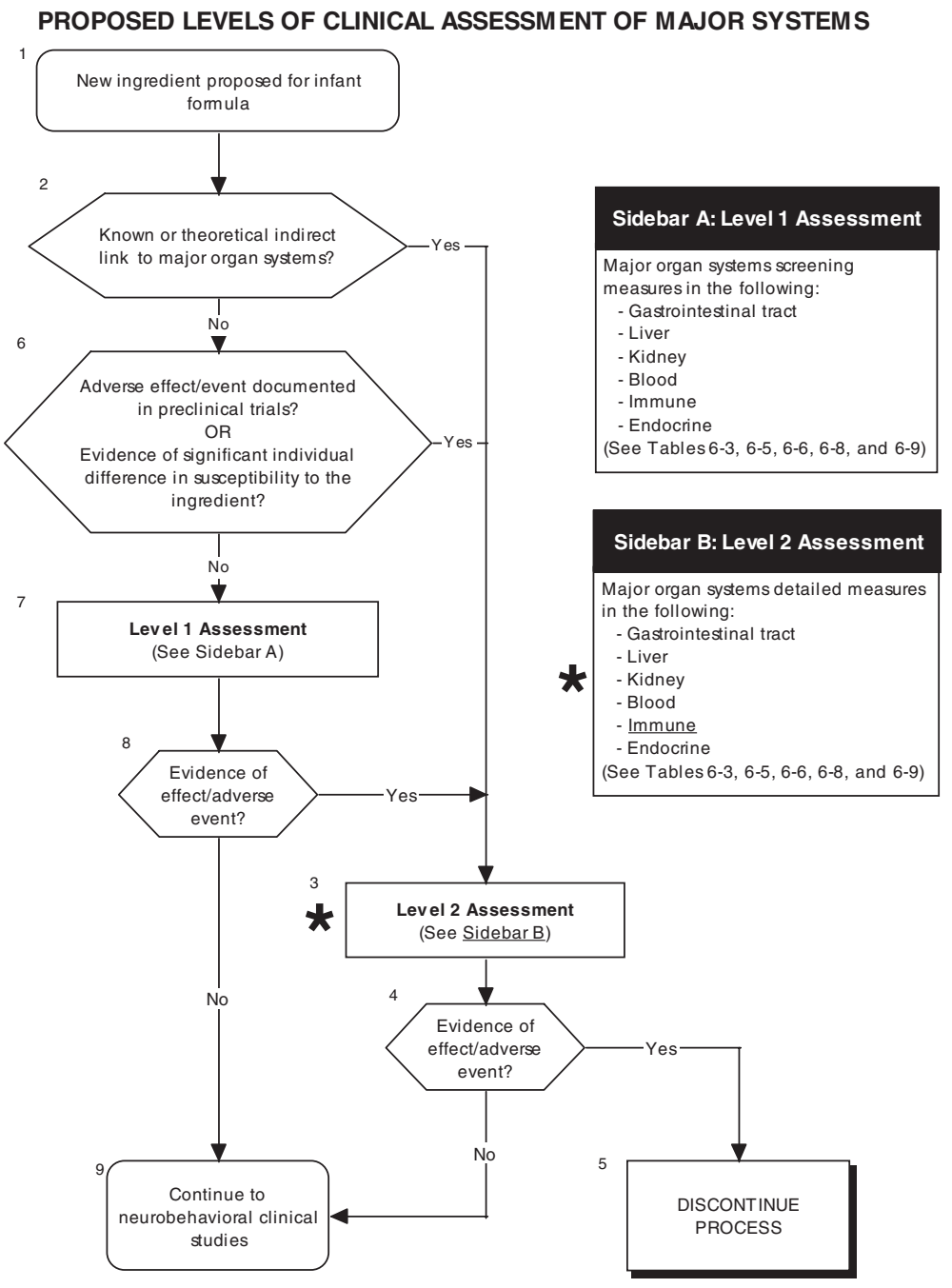

FIGURE D-5 Proposed levels of clinical assessment of major organ, immune, and endocrine systems algorithm: Application by using the long-chain polyunsaturated fatty acid LC-PUFA Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

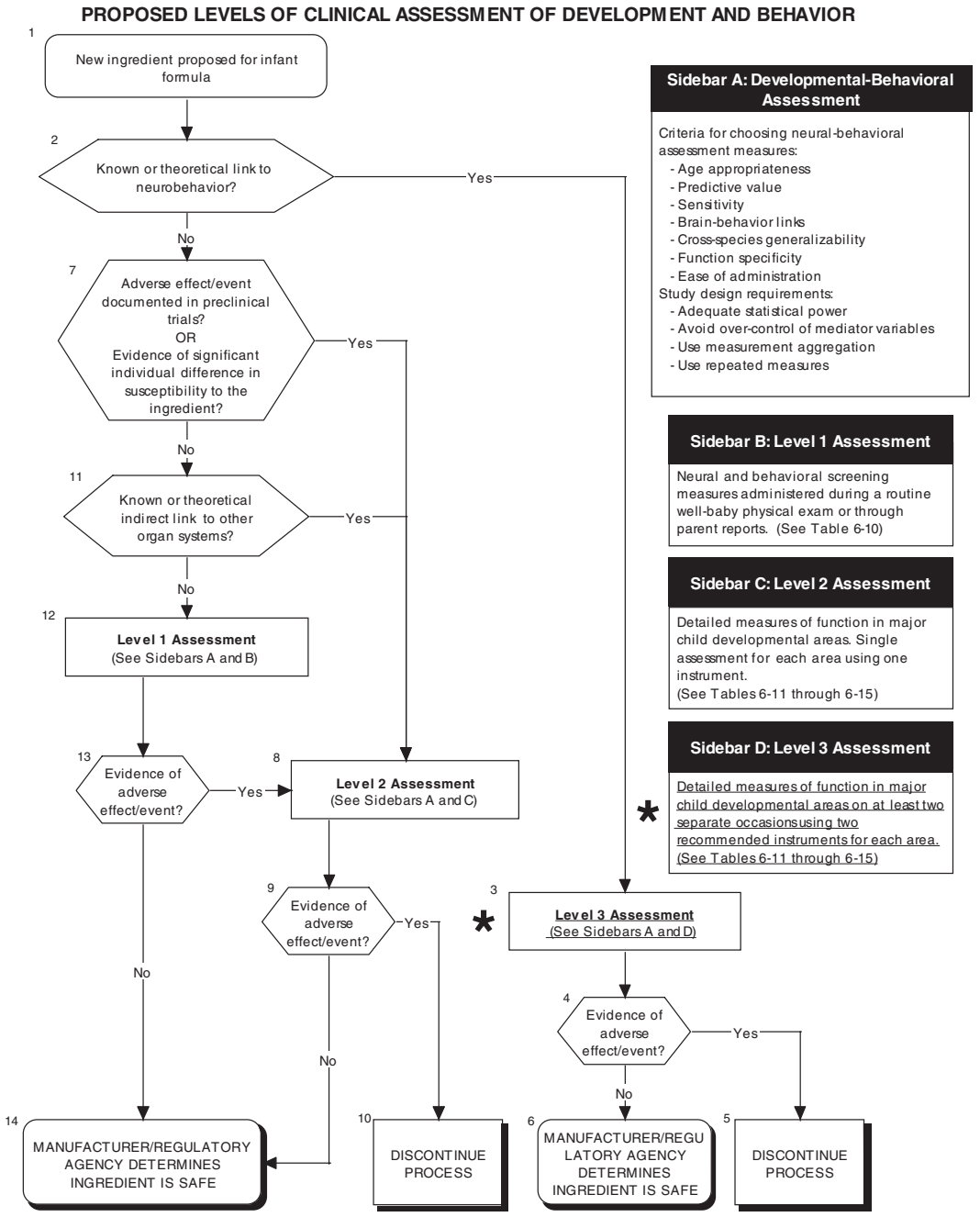

FIGURE D-6 Proposed levels of clinical assessment of development and behavior algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. NOTE: An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

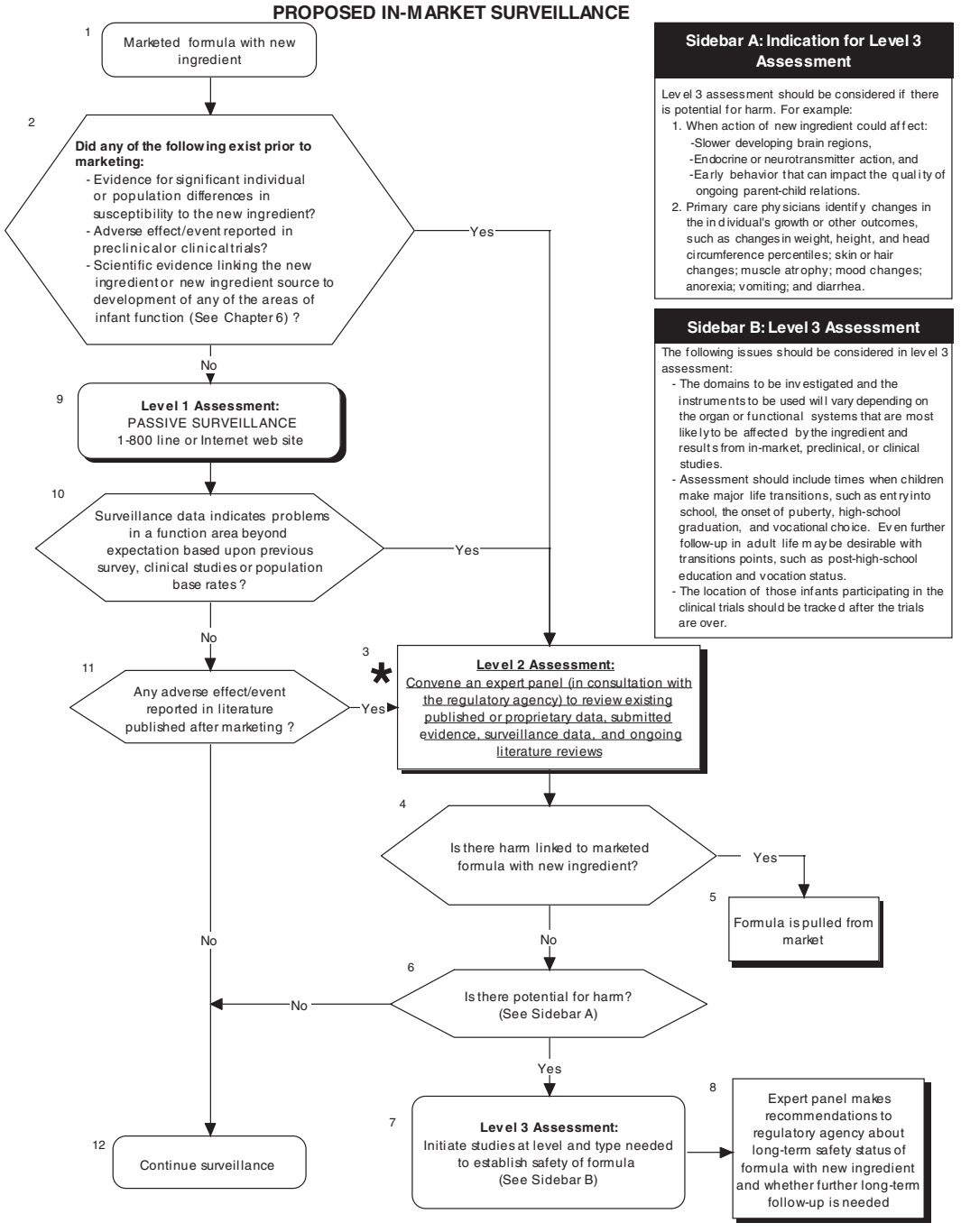

FIGURE D-7 Proposed in-market surveillance algorithm: Application by using the long-chain polyunsaturated fatty acid Generally Recognized as Safe (GRAS) Notifications 000041 and 000080 as a case study. NOTE: An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notifications 000041 and 000080 provided to the committee. ▭ = a state or condition, ⬡ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

of the ingredient. Chapter 7 provides more details on the committee's recommendations for in-market surveillance. If needed, a selection of a qualified and unbiased expert panel is important to evaluate surveillance data and ongoing literature reviews to determine if follow-up studies are necessary.

PROBIOTICS

For the case of probiotics, the committee reviewed GRAS Notice 000049 to analyze the addition of probiotics to infant formula using its recommended algorithms. Figure D-8 provides an overview of the proposed process. Probiotics have a history of safe use in infant formulas in other countries, and a review of the scientific literature showed no significant adverse events linked to these ingredients.

Preclinical Studies

A probiotic is a complex ingredient (e.g., a microorganism) and thus, stability and solubility studies should be performed with the ingredient in solution, as well as in the matrix that would be fed to human infants (Figure D-9). Each probiotic should be tested separately, as well as in the combination that will be used in the infant formula. This is important because different probiotics may possess different chemical characteristics, nutritional contributions, and pharmacological and physiological activities, and they may be derived from novel sources or processes.

A comprehensive preclinical level 1 assessment also should be conducted. As shown in Figures D-9 and D-10, preclinical studies are important in assessing the safety of probiotics since changing intestinal flora may lead to production of atypical components in the intestines. Chapter 5 provides more details on the committee's recommendations for preclinical studies.

Clinical Studies

As seen in the overview of the proposed clinical guidelines (Figures D-11 and D-12), it is important to include appropriate measures of body composition and hepatic and endocrine function. The addition of probiotics could lead to formation of certain molecules at high levels not commonly present in the intestines. This could theoretically affect hepatic and endocrine function and other systems. Finally, clinical studies should include a comprehensive level 1 assessment of behavioral and neural screening measures (see Figures D-11, D-12, and D-13). Chapter 6 provides more information about these assessments.

In-Market Surveillance

Figure D-14 illustrates proposed in-market surveillance guidelines. Assuming that the recommended preclinical and clinical tests using probiotics detected no adverse effects, passive surveillance for in-market monitoring and level 2 long-term follow-up strategies are recommended. Chapter 7 provides more information about the committee's recommendations on in-market surveillance.

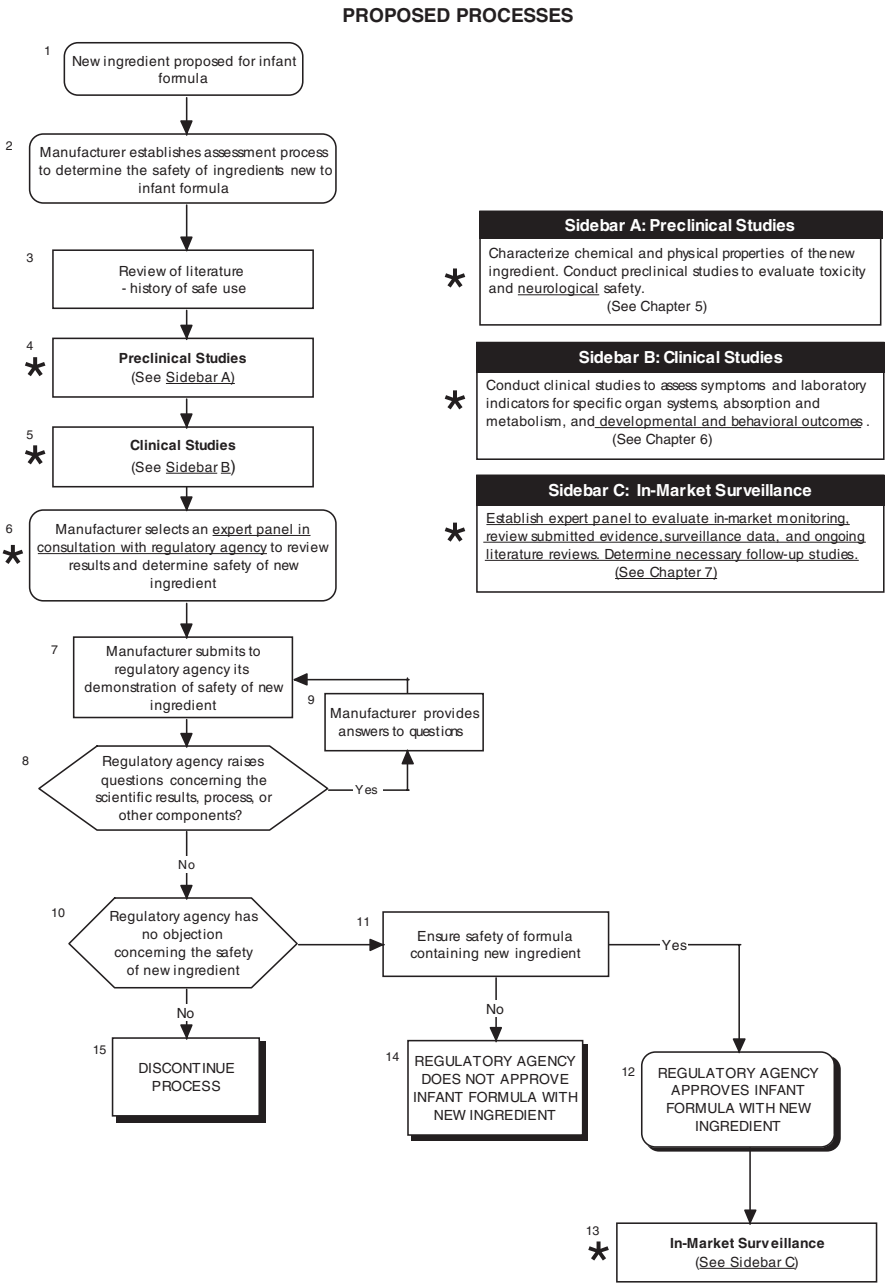

FIGURE D-8 Proposed process for evaluating the safety of ingredients new to infant formulas algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. In-market assessment should be planned in conjunction with preclinical and clinical testing. This algorithm is modeled after the U.S. Generally Recognized as Safe Notification process; similar schemes can be adapted to other regulatory processes. ( ) = a state or condition, ◇ = a decision point, [ ] = an action, sidebar = an elaboration of recommendation or statement.

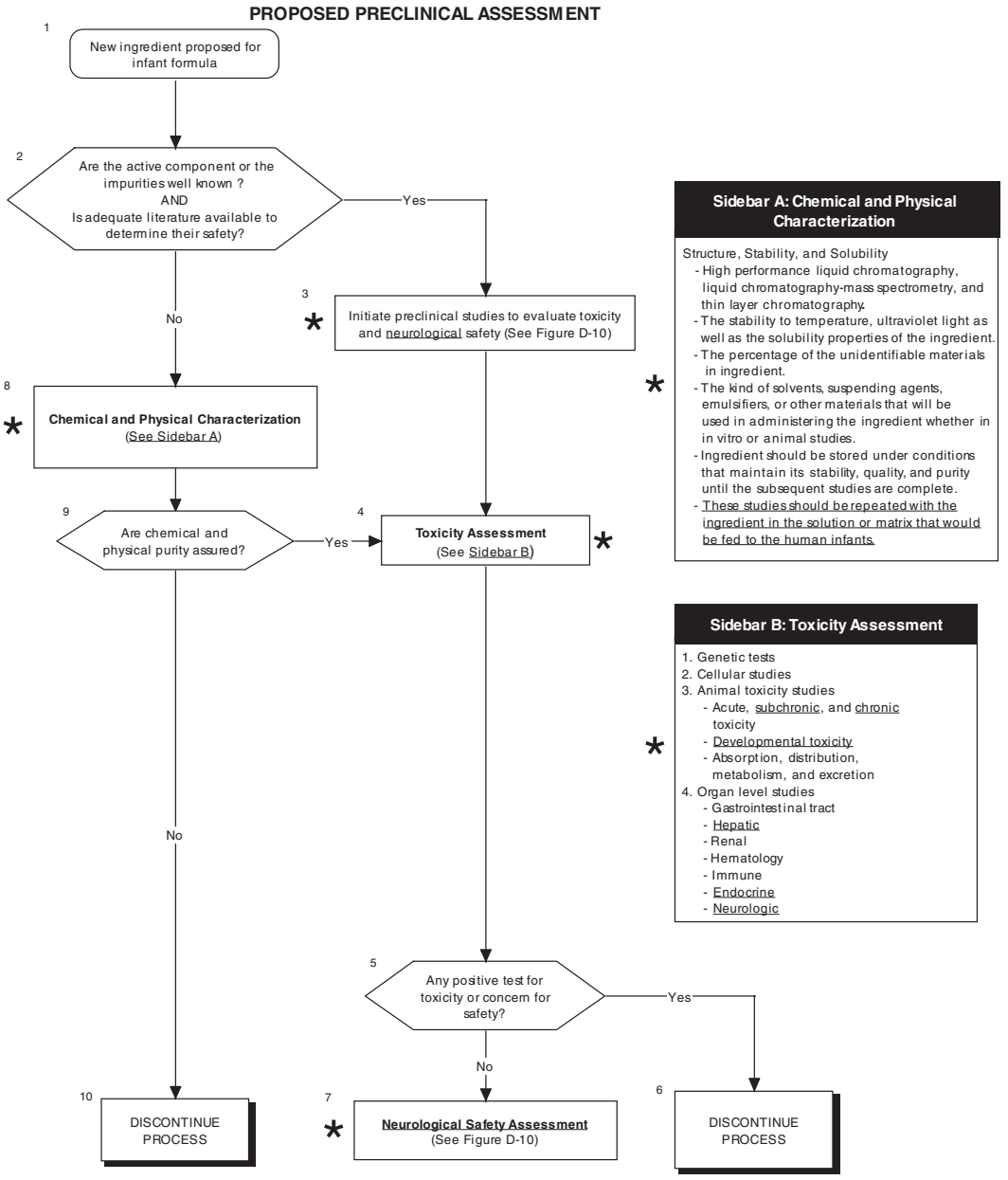

FIGURE D-9 Proposed preclinical assessment algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. Many of the steps of chemical and physical characterization cannot be applied to probiotics. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

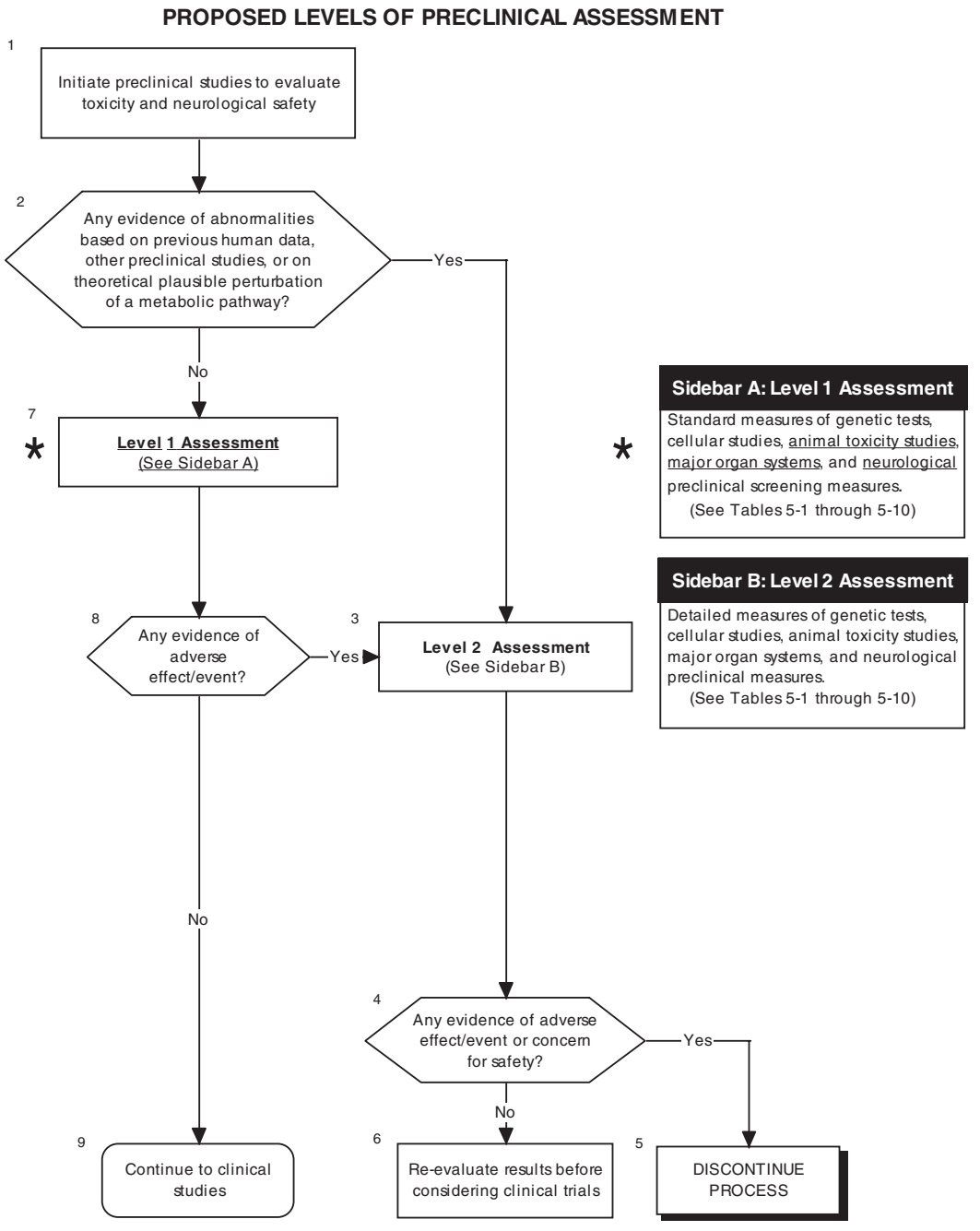

FIGURE D-10 Proposed levels of preclinical assessment algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

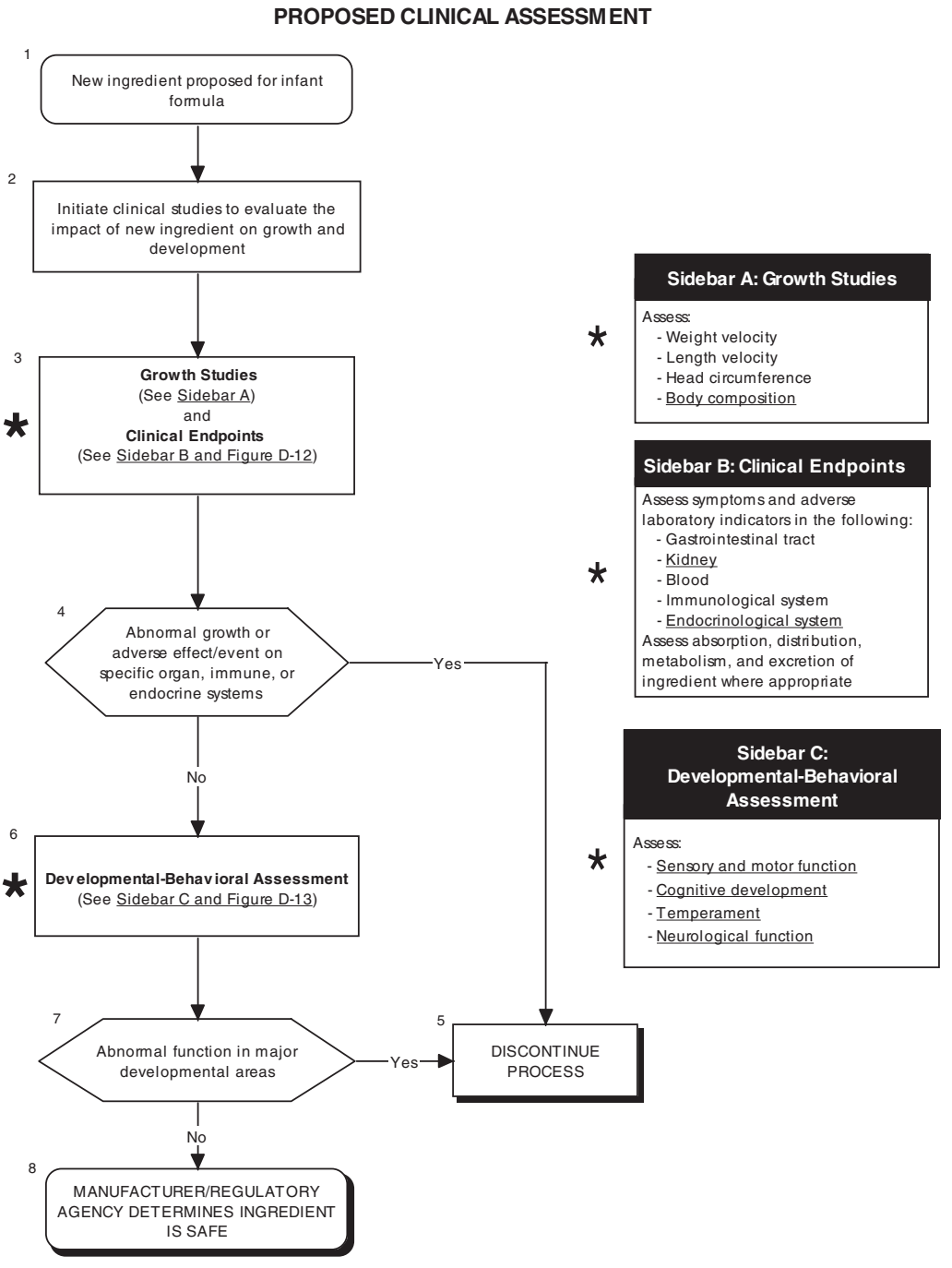

FIGURE D-11 Proposed clinical assessment algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. ( ) = a state or condition, ◇ = a decision point, [ ] = an action, sidebar = an elaboration of recommendation or statement.

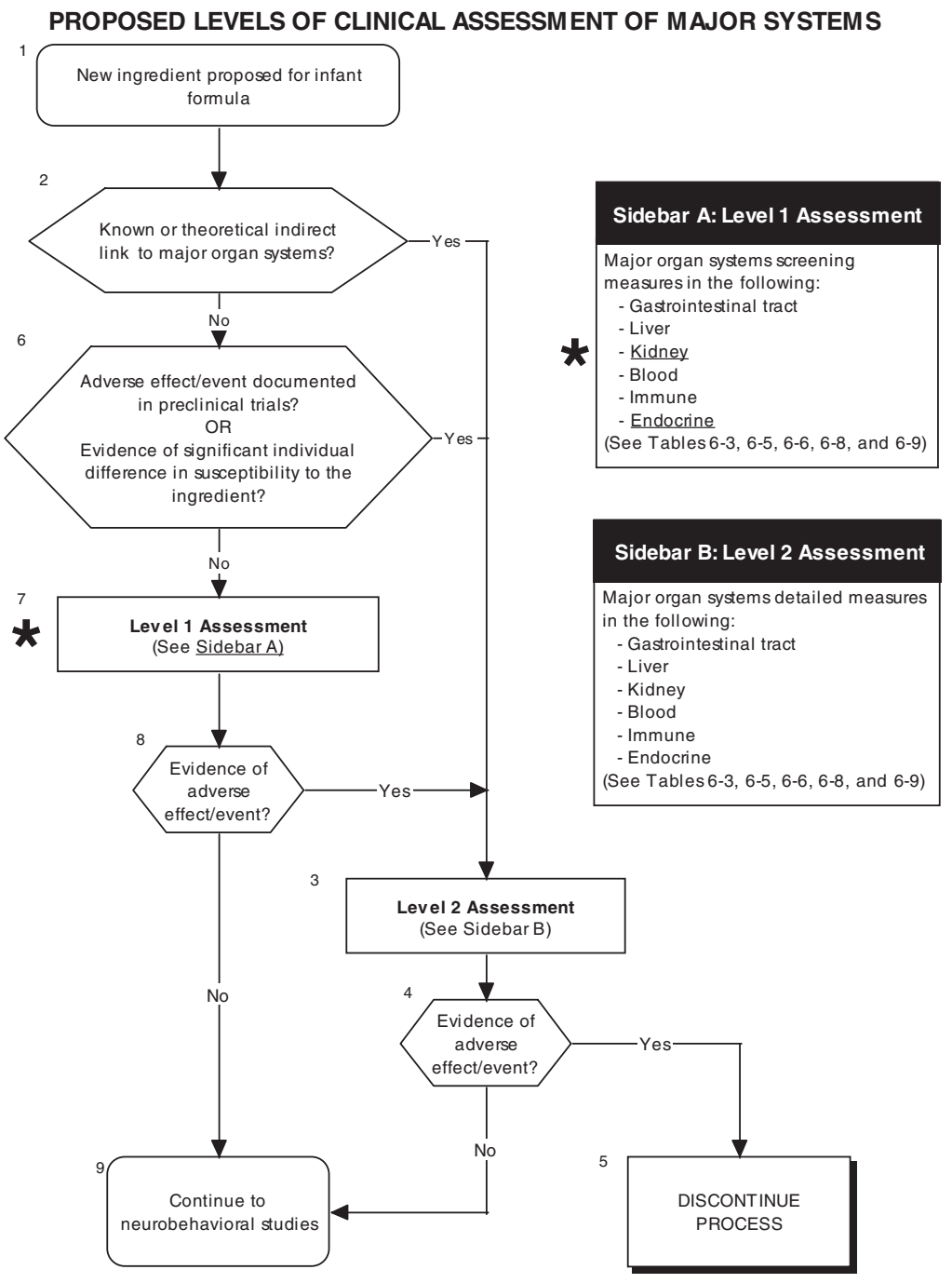

FIGURE D-12 Proposed levels of clinical assessment of major organ, immune, and endocrine systems algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

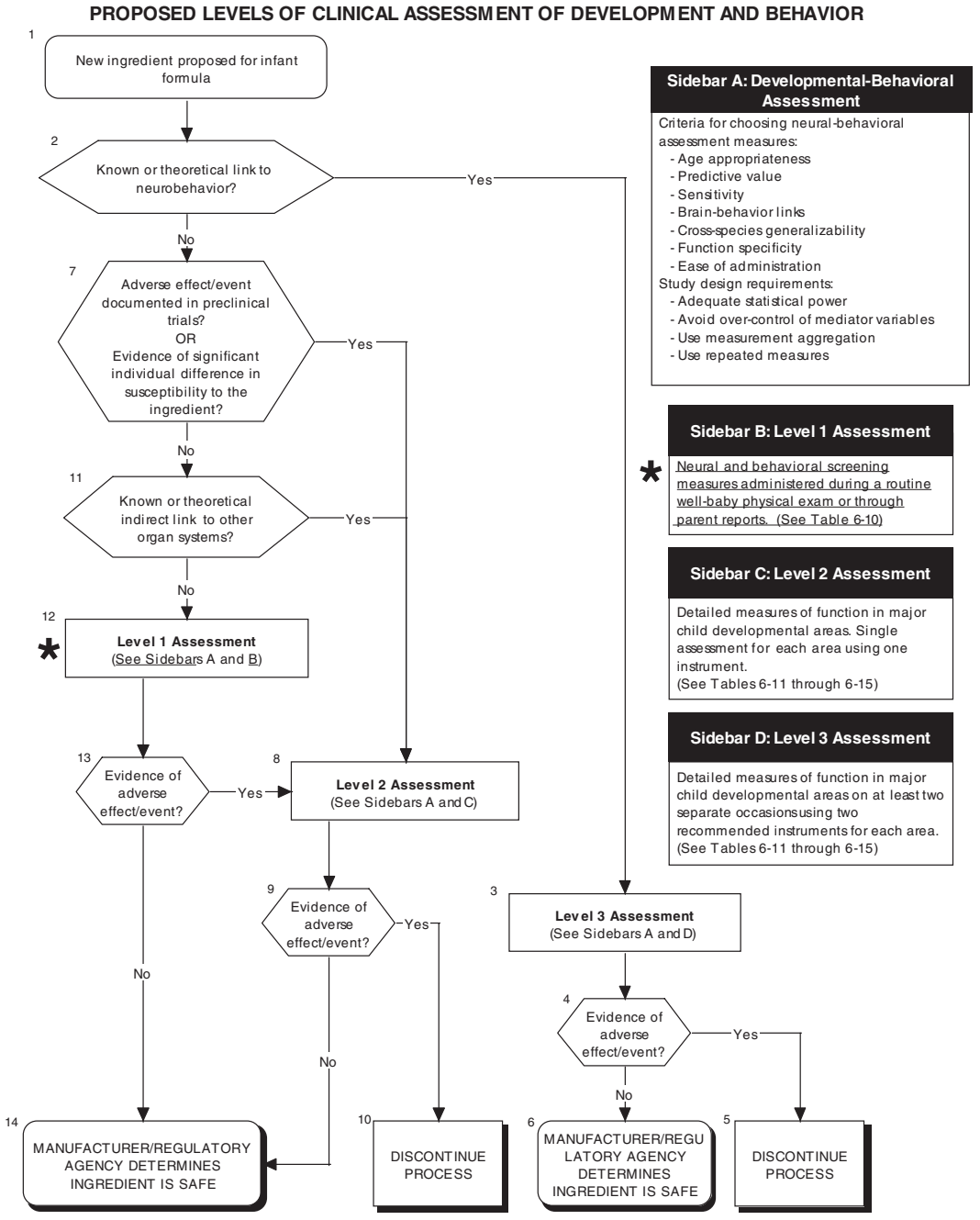

FIGURE D-13 Proposed levels of clinical assessment of development and behavior algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

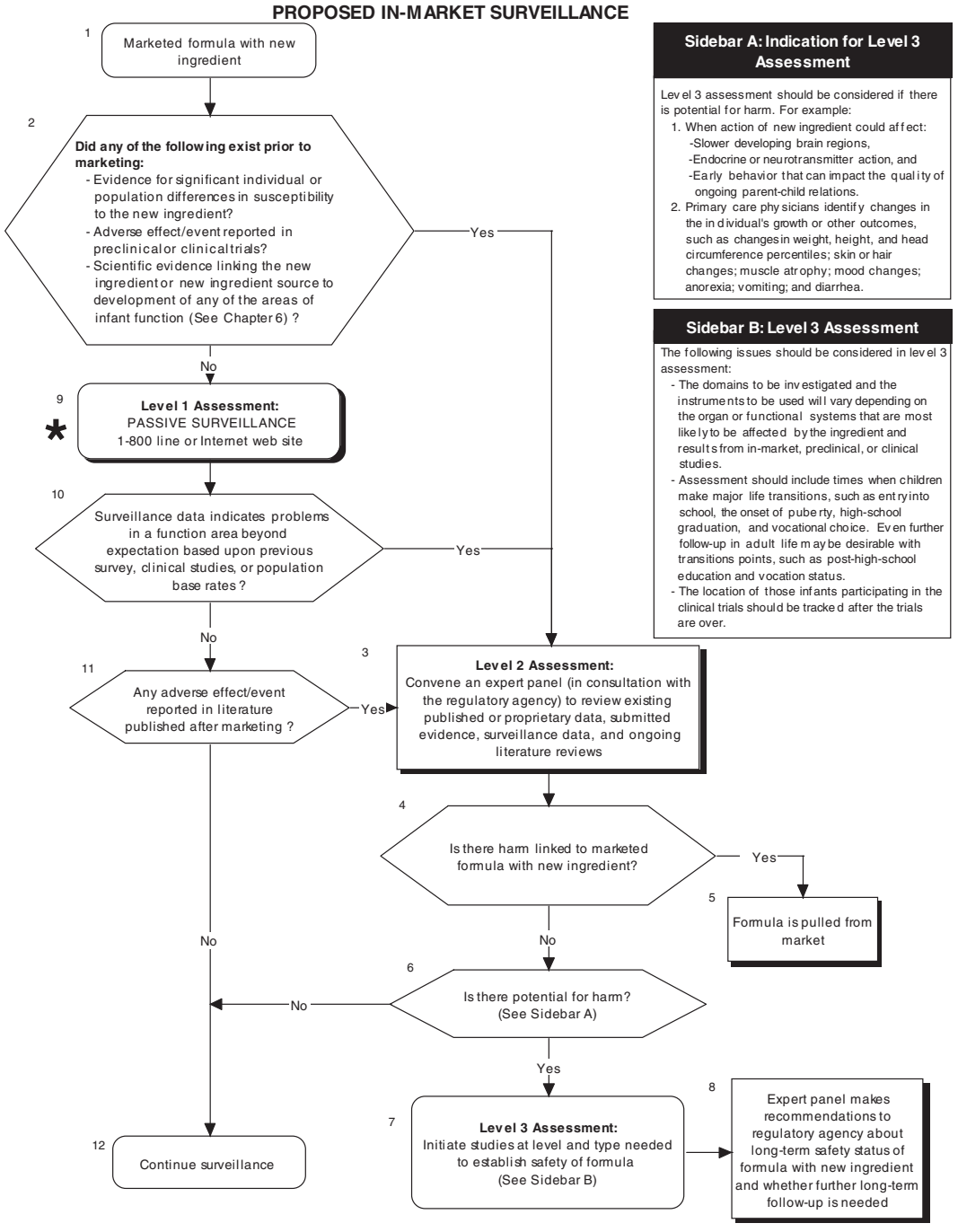

FIGURE D-14 Proposed in-market surveillance algorithm: Application by using the probiotics Generally Recognized as Safe (GRAS) Notification 000049 as a case study. An asterisk (*) along with the corresponding text underlined indicate steps that were either not apparent or not carried out within the GRAS notification 000049 provided to the committee. ▭ = a state or condition, ◇ = a decision point, ▭ = an action, sidebar = an elaboration of recommendation or statement.

SUMMARY

These two case studies demonstrate the flexibility and utility of the proposed processes. They also indicate an important potential role for expert panels in determining the types and levels of assessment to ensure the safety of two very different ingredients. Their application also shows the importance of standardized elements and frameworks when considering the safety of new ingredients added to infant formulas.

E

Biographical Sketches of Committee Members

Richard J. Deckelbaum, M.D. *(chair),* is the Robert R. Williams Professor of Nutrition, a professor of pediatrics, and director of the Institute of Human Nutrition at Columbia University, College of Physicians and Surgeons. His expertise and research interests include regulatory mechanisms for cell-lipid particle interaction and cell cholesterol and triglyceride metabolism. He also coordinates programs related to the effects of varying nutrient intakes on expression of cardiovascular risk factors in children of different genetic backgrounds and the impact of nutritional status on expression of enteric infections. Dr. Deckelbaum received his M.D. from McGill University. He was a member of the U.S. Department of Agriculture/U.S. Department of Health and Human Services' 2000 Dietary Guideline Advisory Committee, chair of the March of Dimes Task Force on Nutrition and Optimal Health, and is a member of the National Institutes of Health Nutrition Study Section.

Linda Adair, Ph.D., is a professor of nutrition at the School of Public Health, University of North Carolina at Chapel Hill, and fellow of the Carolina Population Center. Her research interests include the determinants of early childhood feeding and growth patterns and obesity in childhood and adolescence. She has designed and implemented population-based health, demographic, and nutrition surveys with a special emphasis on longitudinal modeling. She received her Ph.D. from the University of Pennsylvania. Dr. Adair is a member of the Society for International Nutrition Research and the American Society for Nutritional Sciences.

Mark Appelbaum, Ph.D., is a professor in the Department of Psychology at the University of California, San Diego. His research interests involve the application of quantitative and data analytic methods to a wide variety of problems in psychology and the behavioral sciences. Of particular interest to him are the development of quantitative methods for the study of small samples for dealing with problems of variability and for understanding growth and change. Dr. Appelbaum also deals with problems of data structure and analysis in large, multisite studies. He received his Ph.D. from the University of Illinois.

George L. Baker, M.D., retired in 1999 from Mead Johnson Nutritionals where he was vice president and medical director. In this role he provided regulatory, technical, and medical oversight for the launch of new ingredients for infant formulas and drugs. Prior to joining Mead Johnson in 1983, Dr. Baker was associate dean of the College of Medicine and a professor in the Department of Pediatrics at the University of Iowa. Dr. Baker holds an A.B. and an M.D. from the University of Missouri and completed his pediatric residency at the University of Iowa Hospitals and Clinics.

Susan S. Baker, M.D., Ph.D., is a professor of pediatrics at the State University of New York at Buffalo and codirector of the Digestive Diseases and Nutrition Center at Children's Hospital of Buffalo. Her research interests are in pediatrics, general nutrition, and the barrier function of the gastrointestinal tract. Dr. Baker received her M.D. from Temple University School of Medicine and her Ph.D. from Massachusetts Institute of Technology. She recently completed service as chair of the American Academy of Pediatrics Committee on Nutrition and chair of the American Board of Pediatrics Gastroenterology Sub Board. Presently, Dr. Baker is chair of the North American Society of Pediatric Gastroenterology and Nutrition Patient Care Committee.

Cheston M. Berlin Jr., M.D., is a university professor of pediatrics and professor of pharmacology at the Milton S. Hershey Medical Center at the Pennsylvania State University College of Medicine, Penn State Children's Hospital. His expertise and research interests are in pediatric nutrition, lactation, breastfeeding, drugs in human milk, failure to thrive, phenylketonuria, Tourette syndrome, drugs and nutrition, and caffeine. Dr. Berlin received his M.D. from Harvard Medical School. He is a member of the American Academy of Pediatrics, the American Society for Nutritional Sciences, and the American Society for Clinical Nutrition and is chair of the U.S. Pharmacopeia Immunizing Agents Expert Committee.

William C. Franke, Ph.D., is an associate director of the Center for Advanced Food Technology at Rutgers University. He provides technical expertise in the area of product development and food regulations, especially as related to nutraceuticals, and develops new opportunities for technology transfer to small and large companies. He also contributes to administration, marketing, and strategic planning. Previously he spent 28 years at Lipton/Unilever, where he served in a number of senior management positions in product development, quality assurance, and regulatory affairs. Most recently he was Vice President for Scientific and Regulatory Affairs with Unilever United States. He is a member of the boards of the Cancer Institute of New Jersey and the Institute of Food Technologists Foundation.

Michael K. Georgieff, M.D., is a professor in the Departments of Pediatrics and Child Development and codirector of the Center for Neurobehavioral Development at the University of Minnesota School of Medicine. His expertise and research interests are in fetal and neonatal nutrition and neurodevelopment, with special emphasis on the effect of fetal/neonatal iron nutrition on brain development and neurocognitive function and the effect of illness on neonatal protein-energy metabolism. Dr. Georgieff received his M.D. from the Washington University Medical School. He is a member of the Perinatal Research Society, the American Academy of Pediatrics, the Society for Pediatric Research, and the American Pediatric Society.

James M. Ntambi, Ph.D., is the Steenbock Professor in the Department of Biochemistry and Nutritional Sciences at the University of Wisconsin, Madison. His expertise and research

interests are in the genetic regulation of lipid and carbohydrate metabolism. Dr. Ntambi's experimental work on the genetic regulation of the stearoyl-CoA desaturase enzyme has recently led to many new insights into the importance of this enzyme in metabolism and in disease states, such as obesity, diabetes, atherosclerosis, and cancer. His pioneering work will help to explain the complex aspects of the "Metabolic Syndrome" and to advance our understanding of nutrient-gene interactions. Dr. Ntambi serves on several university committees and National Institute of Health study sections and is a member of the National Institute on Alcohol Abuse and Alcoholism's Board of Scientific Counselors. Dr. Ntambi received his Ph.D. from Johns Hopkins University School of Medicine and is a member of the American Society for Nutritional Sciences.

Theodore D. Wachs, Ph.D., is a professor of psychological sciences at Purdue University. His research interests are in two major areas: the role of early physical and social environments upon subsequent development and the relation of chronic, mild nutritional deficits to infant and toddler cognition, temperament, and parent-child relations. Dr. Wachs received his B.A. from Muhlenberg College and his M.S. and Ph.D. in clinical psychology from George Peabody College. He is a member of the Society for Research in Child Development and the International Society for the Study of Behavioral Development and is a fellow of the American Psychological Association.