reference standards allow researchers and reviewers to identify those infants whose growth is not following expected longitudinal patterns and, therefore, for whom a more thorough review of their medical and dietary histories is necessary to assess the possibility that the infant formula is responsible for reduced growth rates in a subgroup of infants. This careful review of individual infant growth patterns in addition to group summary data is particularly important because studies, while adequate to evaluate differences in group means between test and control formulas, often lack the statistical power to detect subgroups of infants whose growth patterns deviate from normal. These data will also provide useful information on possible trends towards failure to thrive or obesity, or on catchup growth in infants who experienced transient adverse effects relative to expected growth rates.

FDA has tentatively concluded that a comparison of a manufacturer's data to well-established population reference standards can provide the basis for an evaluation of the growth patterns of individual infants to identify, and to provide the basis for an investigation of, possible causes of unusually slow or fast rates of gain. Thus, the agency is proposing that the NCHS growth charts for individuals and for grouped data be incorporated by reference into the regulation (proposed § 106.97(a)(1)(i)(B)).

Proposed § 106.97(a)(1)(i)(C) requires that the manufacturer collect the anthropometric measurements at the beginning of the clinical study, at 2 weeks and at 4 weeks of the study, at least monthly thereafter, and at the conclusion of the study. These measurements will permit the calculation of incremental gains in the different measurements. Incremental gains, such as weight gain per unit of time, are generally considered the most sensitive indicator of the ability of a formula to support the physical growth of individual infants over time (Ref. 4). Also, because growth rate and nutritional requirements are curvilinear rather than linear during early infancy, multiple measurements help in assessing whether the formula meets the nutritional needs throughout the period of the clinical study and aids in more accurately placing infants in their "correct" reference percentile tract, particularly since age of enrollment varies somewhat among infants (although, if adopted, this regulation should serve to minimize that variation). Additionally, measures of an infant's body weight, the most critical anthropometric measure, are subject to a number of measurement errors unrelated to the nutritional value of the formula (e.g., timing of weighing of infant relative to feeding or defecation or urination).

For these reasons, multiple measures over a relatively long period (e.g., 4 months) provide a more accurate picture of the pattern of growth of infants than do one or two point measures. The agency has tentatively concluded that the requirement of four measurements taken 1 month apart will provide a sufficient number of measurements to permit evaluation of whether the formula meets the nutritional needs for physical growth of the infant throughout the study period. However, the agency requests comment, supported by data, on which measurements are needed to provide evidence that the formula meets the nutritional needs for physical growth of infants.

FDA has tentatively concluded that more frequent measurements are needed during the early stages of the study because variations in measured body weight that are a result of factors unrelated to the nutritional quality of the formula can be particularly serious in early infancy. For example, during the first week of life, there is a normal loss of body weight by the infant because of fluid loss that may reach 6 to 10 percent of body weight (Ref. 76). This weight loss will reduce the apparent growth of the infant as measured by body weight. This reduction may affect the ability to evaluate and interpret the weight gain data collected early in the study. FDA has tentatively concluded that requiring more frequent anthropometric measurements, especially for weight, early in the study, increases the ability to accurately place individual infants in the correct percentile track for monitoring their growth patterns in relation to the population reference curves and for monitoring physical growth during the most sensitive part of their growth phase.

To minimize the burdens of this regulation, FDA has not proposed to require that blood samples obtained from infants during the time period of their enrollment in the clinical study, or at completion of the study, be analyzed for biochemical and clinical indicators of nutritional and growth status. However, the CON/AAP Task Force (Ref. 6) recommended that some blood tests be conducted at the conclusion of required clinical studies to provide a more comprehensive evaluation of the nutritional adequacy of a formula. Thus, the agency requests comments on whether it would be useful for the manufacturer to collect and maintain data on standard laboratory measures, including complete blood count (white blood cell count and red blood cell count), hemoglobin concentration or hematocrit percentage, and serum or plasma concentrations of albumin, urea nitrogen, electrolytes (sodium, potassium, and chloride), alkaline phosphatase, and creatinine. These measurements are standard practice when infants are seen clinically and can be made with very small quantities of blood. The maintenance of these indicators within normal limits at the end of the study provides additional assurance over and above measures of physical growth that the infant's general state of well-being is healthy and "normal," particularly because changes in biochemical measures may occur before detectable differences in physical growth are identified or may not be detected by measures of physical growth. General anthropometric measurements of physical growth provide indirect, although very important, evidence that the formula is able to help the infant maintain overall good health, but they are not as specific, and may not be as sensitive, as are biochemical indicators of health.

FDA also requests comment on whether requiring some, or all, of the biochemical and clinical tests described above would provide useful and necessary information for determining whether a formula causes adverse consequences that may not be reflected in the quality factor requirements for measurements of physical growth in proposed § 106.97(a)(1)(i).

The identification of deviations from expected values for these biochemical and clinical measurements, throughout the duration of the clinical study, could serve as an early warning of unexpected risk to infants enrolled in the study and, therefore, result in early actions to prevent undue risk to infant enrollees in the study. Conversely, collection of blood samples throughout the study could discourage parents from continuing their infants in the study, thus causing a high attrition rate and producing final study results that are difficult to interpret.

Proposed § 106.97(a)(1)(ii) sets forth guidelines for the design of clinical study protocols. A comprehensive clinical study protocol will ensure that individual investigators understand and follow generally accepted scientific principles for the design and conduct of clinical trials, thus enhancing the likelihood of interpretable results while maintaining minimal or no risk to infants enrolled in the studies. In the conduct of all studies, manufacturers should use the general principles,

described in § 314.126 (21 CFR 314.126) for adequate and well-controlled clinical studies to ensure that the design and conduct of the study are adequate to permit scientific review and interpretation of the study's results. Studies that cannot produce meaningful results because of poor or inadequate study design and conduct mean that infants will be subjected to unnecessary testing. Such a situation places infant enrollees at undue risk and is clearly unethical.

In this section, FDA is not establishing mandatory elements for inclusion in a protocol, nor requiring that manufacturers provide the agency with the protocol used for a study intended to provide data to show that an infant formula meets the quality factor requirements. However, as discussed above, a protocol is an essential part of the design and execution of a well-controlled scientific study. Furthermore, a protocol often provides invaluable information that assists in the analysis and interpretation of the study data. Consequently, the agency strongly encourages manufacturers to develop and use protocols that incorporate the specific elements in proposed § 106.97(a)(1)(ii) in all research studies using infants because these elements will ensure that the study is designed and conducted in a manner that will produce results that will permit meaningful evaluation of the usefulness of the infant formula.

The steps outlined in proposed § 106.97(a)(1)(ii)(A) represent standard practice in the design and conduct of clinical studies (Ref. 72). Proposed § 106.97(a)(1)(ii)(B) states that the clinical study protocol should describe the necessary qualifications and experience of the investigators. It is essential that clinical studies be conducted by personnel with sufficient experience and training to ensure that their work will yield interpretable and meaningful results. If a study is conducted by an investigator who is not qualified, it increases the likelihood that the study will have to be redone, and that more infants will be exposed to risk. Therefore, it is important that in the protocol, the manufacturer define the requisite qualifications to conduct the study it is designing.

Proposed § 106.97(a)(1)(ii)(C) states that the protocol should be reviewed and approved by an Institutional Review Board (IRB) in accordance with part 56 (21 CFR part 56), and that the manufacturer should establish procedures to obtain written informed consent from the parents or legal representatives of the infants enrolled in the study in accordance with part 50 (21 CFR part 50). These steps are necessary to protect the rights and safety of subjects involved in the studies.

Proposed § 106.97(a)(1)(ii)(D) states that the clinical study protocol should explain how the study population represents the population for which the new infant formula is intended. FDA has tentatively concluded that such an explanation is necessary so that if questions about the relevance of the study population arise, the answer is readily available and free of any taint that it is a post hoc rationalization. For example, FDA has recently had questions about a study that involved hospitalized infants that were offered to support use of the product on post-discharge infants. If there had been the type of explanation available that FDA is proposing in this guideline, it would have greatly minimized the questions about this product.

Proposed § 106.97(a)(1)(ii)(D) also states that the clinical study protocol should explain how the study addresses the intended conditions of use of the formula. FDA has tentatively concluded that, by having manufacturers consider this question before the study is conducted, this guideline will prevent clinical studies that are conducted under conditions of use that do not accurately reflect the proposed conditions of use. For example, a clinical study protocol for testing a formula designed to be used by premature infants throughout infancy should explain how the study design will provide information to support the claim that the formula supports healthy growth under these conditions.

Proposed § 106.97(a)(1)(ii)(E) states that the clinical study protocol should describe the sample size calculations and the power calculations and the basis for selecting the sample size and study design. This information is necessary to establish the likelihood that the study will not fail to detect a real difference, should there be a difference for the measurements of interest, between the infant formula being tested and the control. For example, a study might not find a difference in incremental rate of weight gain between infants consuming two formulas because too few infants were enrolled in the study to provide sufficient statistical power to detect this difference. Inadequate statistical power could mask the nutritional inferiority of a product and could result in the marketing of a formula that does not meet the quality factor requirements and, therefore, is not safe for its intended use. Therefore, FDA has tentatively concluded that this guideline is needed to ensure that manufacturers design their growth studies to be capable of detecting biologically meaningful differences for the endpoints of interest between the two formulas. Identification of differences would raise safety concerns or serious questions of nutritional quality of the test formula product.

Proposed § 106.97(a)(1)(ii)(F) states that the clinical study protocol should include a plan to identify and evaluate any adverse events. This proposed guideline is necessary to document that appropriate attention is given to the systematic evaluation and recording of any adverse events that may occur during the course of the study. Inadequate planning for and conduct of the monitoring of adverse events may result in an erroneous conclusion that the formula is safe and suitable, when in fact the formula is not safe and suitable for infants under intended conditions of use.

Proposed § 106.97(a)(1)(ii)(G) states that the clinical study protocol should describe the quality control procedures that the investigator will use to ensure the validity and reliability of the measurements collected. This proposed guideline represents standard practice in the design and conduct of clinical studies and is necessary to allow a meaningful interpretation of study results. Data obtained with unreliable measures, or with indicators that do not accurately or meaningfully measure identified endpoints, may produce misleading study results that are uninterpretable and that suggest that a formula is safe and suitable, when more valid or reliable measures would not have supported this conclusion. The institution of adequate quality control procedures before beginning a study provides a mechanism for manufacturers to ensure that the data collected are reliable, and that the study provides interpretable results.

Proposed § 106.97(a)(1)(ii)(H) states that the clinical study protocol should describe and compare the composition of the control and test formulas. These descriptions of the control and the test formulas are necessary to establish that the formula used as the control provides an adequate comparison for evaluating the quality factors of the test formula. If the control formula is not comparable to (i.e., bioequivalent to) formulas in current use, differences between the test and control formulas have no meaning. They cannot be generalized to projected conditions of use. For example, comparable or enhanced physical growth in infants consuming a test formula as compared to infants consuming a control formula when the control formula does not meet

requirements in § 107.100 for nutrients, or is not bioequivalent relative to quality factors to currently marketed formulas in the United States, cannot be interpreted as supporting healthy growth because it is not possible to determine whether the apparent "equal" or "enhanced" physical growth is attributable to the fact that the formula is nutritionally adequate, or whether the formula looks adequate because it is being compared to a nutritionally inadequate formula. The nature of the differences between control and test formulas will also affect sample size and measurement (endpoint) considerations.

FDA's experience in reviewing clinical data submitted with 90-day notifications has been that the absence of information on control formulas is not uncommon. Thus, FDA has tentatively concluded that a guideline on the information that needs to be considered in selecting a control formula is necessary to ensure that study results are meaningful and interpretable.

If the test formula used in a study is not identical to the formula that is intended to be marketed in the United States, proposed § 106.97(a)(1)(ii)(I) states that the clinical protocol should describe the basis upon which the manufacturer has decided that the test formula is appropriate for use in the study. This proposed guideline is necessary to ensure that the manufacturer considers such factors as the bioequivalence of the studied (test) formula relative to the formula that is to be marketed in this country and can document why its choice of test formulas is appropriate. Without this documentation, it would not be possible to determine whether the marketed formula meets the quality factor requirement in proposed § 106.96(b).

FDA has had experience under the 1986 amendments in which manufacturers have submitted data on test formulas that were significantly different (e.g., in calorie levels) from the formula that they intended to market as evidence of the safety and suitability of the latter formula. In these instances, the agency has had considerable difficulty in interpreting study results. Therefore, if the guidance in proposed § 106.97(a)(1)(ii)(I) is followed, this significant study design issue will be critically reviewed by manufacturers before they initiate their studies, and, as a result, they will be more likely to design and conduct a study that will produce data that can be meaningfully interpreted as evidence that an infant formula is safe, and that it supports healthy growth.

As provided in proposed § 106.97(a)(2), however, FDA recognizes, that while changes in ingredients or in the processes used in the manufacture of infant formulas can have a significant adverse impact on the levels or availability of nutrients that affect healthy growth of infants, other changes may not be likely to do so. In the latter circumstances, it may be possible to demonstrate that the quality factor requirements are met by means of measures or data that do not involve the use of clinical trials. If such assurances can be provided without clinical trials, then infants will not be subjected to unnecessary testing. Therefore, FDA sets out in proposed § 106.97(a)(2), the circumstances in which a manufacturer can request an exemption from the clinical study requirement.

Proposed § 106.97(a)(2)(i) provides for an exemption if the manufacturer can cite experience that shows that the ingredient, ingredient mixture, or processing method has been used to make an infant formula that meets the quality factor requirements in proposed § 106.96(a). For example, if the manufacturer has previously submitted information to FDA in response to the quality factor requirements of the act that showed that an infant formula that contains the ingredient or ingredient mixture, or that was produced by the processing method, in question supported adequate physical growth, this information could form the basis on which the new infant formula could qualify for an exemption from this quality factor requirement. Under this provision, FDA will evaluate the experience cited in support of an exemption on a case-by-case basis. FDA requests comment on this proposed provision.

Proposed § 106.97(a)(2)(ii) provides for an exemption if a manufacturer that markets a formulation in more than one form (such as liquid and powdered forms) can demonstrate that the quality factor requirements are met by the form of the formula that is processed using the method that has the greater potential for adversely affecting the formula's nutrient content and bioavailability. For example, the temperatures used to retort liquid formulas during processing can cause a loss of protein quality compared to powdered forms processed at lower temperatures (Refs. 77 and 78). Thus, if the liquid formula is tested and shown to meet the quality factors requirements, it will provide reasonable assurance that the powdered form of the formula, that is, the less processed form is of appropriate nutritional quality. Thus, FDA tentatively concludes that it would be unnecessary to test the less processed form.

Proposed § 106.97(a)(2)(iii) provides for an exemption if the manufacturer can demonstrate that the requirements of proposed § 106.97(a)(1) are not appropriate for the formula, and an alternative method or study design for showing that the formula supports healthy growth in infants fed the formula as a sole source of nutrition is available. As stated above, double-blind, well-controlled, clinical studies are generally the most powerful and sensitive method for demonstrating that an infant formula will support physical growth. Nonetheless, the agency anticipates that there will be circumstances in which a clinical study of a new infant formula would not be appropriate. For example, double-blind clinical studies would not be appropriate in situations such as those involving some exempt infant formulas in which they would cause withholding of conventional treatment and, therefore, would be unethical. Other situations that may not be amenable to double-blind clinical trials are those in which it would be difficult to enroll an adequate number of infants (e.g., for exempt infant formulas where the formula is intended for a rare disease). Alternative study designs may also be appropriate in situations in which a manufacturer has access to extensive reference data, such as a database on many similarly conducted clinical studies using infants from the same potential study population, provided that the manufacturer can demonstrate that the reference data apply to the new infant formula, its intended use, and its study population. FDA has tentatively concluded that such an exemption will permit flexibility in the design of suitable experimental protocols but still provide reasonable and documentable assurance that the study design can demonstrate the safety and suitability of the infant formula.

b. *Specific quality factors.* Proposed § 106.97(b) establishes requirements for demonstrating that a formula meets the protein quality factor requirement in proposed § 106.96(c) and requires that the manufacturer collect and maintain data that establish that the biological quality of protein in an infant formula is sufficient to meet the protein requirements of infants by demonstrating that the protein source supports adequate growth using the PER rat bioassay, which the agency proposes to incorporate by reference. The PER provides an estimate of the bioavailability and relative proportion of the essential amino acids in the protein-containing ingredient.

A chemical analysis of the protein can identify the amino acids contained in a protein source but does not measure their bioavailability. A protein source may contain the necessary amino acids, but they may be in a form that the infant cannot digest and absorb. Furthermore, processing methods may alter the chemical nature of the protein source, possibly making the protein more resistant to digestion by the infant. FDA has tentatively concluded that the rat bioassay is necessary to establish that the amino acids in a protein source are present, and that adequate amounts and proportions of all essential amino acids are capable of being digested by an infant. Such a showing is particularly important when a manufacturer is using a novel protein source (e.g., a hydrolyzed protein), a new protein mixture, a new processing method that could affect the chemical form or bonding of amino acids, or a formulation that provides an amount of protein near the minimum required level (<2.0 g/100 kilocalorie (kcal)) specified in § 107.100.

Proposed § 106.97(b)(1) also provides that if the manufacturer is unable to conduct a PER rat bioassay, it must demonstrate that the amino acid composition of the protein meets the known amino acid requirements of infants for whom the formula is intended. For example, FDA is aware that a PER would not provide useful data for an exempt infant formula intended for use in infants that cannot metabolize a specific amino acid and from which that amino acid has been purposefully omitted or is limited to a level inadequate to support healthy growth. The lack of that amino acid is necessary for the dietary management of the intended infant population but would result in an incomplete protein and would reduce the growth rate of the rat, invalidating the conditions upon which the PER rat bioassay is based. FDA is not aware of alternative methods for ensuring bioavailability of such a protein source. In these circumstances, proposed § 106.97(b)(1) will provide an alternate means of evaluating whether the protein at least contains adequate amounts of essential amino acids to meet the known amino acid requirements of the infant, even though the bioavailability of these amino acids cannot be assured using available methods.

Proposed § 106.97(b)(2) establishes the circumstances in which a manufacturer may request an exemption from the requirements of proposed § 106.97(b)(1). Proposed § 106.97(b)(2)(i) provides that if the protein source (including the processing method used to produce it) is already used in another of the infant formulas marketed by its manufacturer in the United States, the manufacturer may request an exemption if it can demonstrate that such other infant formula meets the quality factor requirements prescribed in § 106.96(b)(1). The purpose of the PER or amino acid analyses is to estimate the quality of the protein in the proposed formula. Once a manufacturer has established standard sources and processing of protein in a formula, and has demonstrated that the technology is effective, in its hands, in producing a formula that meets the quality factor requirement for protein, other formulation changes would not be expected to markedly affect protein quality. Thus, the quality of the processed protein would be retained in other formulas. However, under proposed 106.97(b)(2)(i), it will be incumbent on the manufacturer to demonstrate that the quality of the protein is not affected.

Proposed § 106.97(b)(2)(ii) provides for an exemption if the protein source, or the processing method used to produce the protein source, in the infant formula does not constitute a major change from the infant formula that it replaces, and the manufacturer can demonstrate that the infant formula that it replaces meets the quality factor requirements prescribed in § 106.96(b). FDA is proposing to allow this exemption because it is unlikely that the methods for assessing protein quality prescribed are sensitive enough to measure any change in protein quality that is not a major change.

Because FDA has, as a matter of policy, been requesting that infant formula manufacturers submit data from a PER or amino acid analysis as part of their submission 90 days prior to marketing infant formula, many infant formulas that are on the market have been shown to meet the proposed quality factor requirement for protein. Therefore, if the proposed exemption criteria in § 106.97(b)(2) are adopted, those formulas that contain protein sources, or proteins which were produced using processing methods, that were the subject of a submission to FDA in response to the quality factor requirements of the act may qualify for an exemption.

6. Request for Comment on Establishing Assurances for Other Quality Factors

As discussed above, FDA has solicited comment on whether to establish quality factor requirements for fat, iron, and calcium and phosphorus. If such quality factors are adopted, appropriate methods will be needed to provide assurance that an infant formula meets these nutrient-specific quality factors. Therefore, FDA discusses below measurements of fat balance and of calcium and phosphorus balance, as well as measurements that reflect iron bioavailability. The agency requests comments and information on these or other methods for these three quality factors:

a. *Apparent fat absorption.* Apparent digestibility and apparent absorption measure the amount of fat that was able to be digested and absorbed by the infant. Apparent digestibility is expressed as a percentage of intake, while apparent absorption is expressed in units of fat (e.g., g) absorbed per day. If a quality factor for fat were established, manufacturers would be required to collect and maintain data establishing that the apparent digestibility or apparent absorption by the infant of the fat in an infant formula is adequate to meet the infant's energy requirements. These data would be necessary because fat represents the major dietary source of energy for the infant and must be readily digested and absorbed if the formula is to support healthy growth.

The CON/AAP Task Force (Ref. 6) recommended that studies that are conducted to determine whether a formula meets the quality factor for fat should use a cross-over experimental design. This type of study requires that the manufacturer compare apparent fat absorption of infants fed the test formula at one time and a currently marketed formula at another time. An experiment using this design would enable a manufacturer to make measurements of apparent fat absorption using a small number of infants, since the variance in fat excretion of infants fed most fat sources currently available is less than 5 percent. Furthermore, the method is noninvasive, is easily implemented, and does not require costly or sophisticated equipment to conduct. Other experimental designs could be used but would require larger numbers of infants and would be more expensive. Thus, FDA asks for comments on whether there should be a specific requirement that manufacturers measure apparent fat absorption using cross-over studies.

The CON/AAP Task Force (Ref. 6) recommended that studies that are conducted to determine the apparent absorption of fat be conducted such that measurements are made using infants fed each formula for at least 72 hours. The Task Force report suggested that measurements of apparent fat absorption for this length of time would accurately reflect the apparent

absorption of the fat in the formula being tested. FDA is considering requiring that a study of at least 72 hours for each formula tested be conducted and requests comment on what duration would be appropriate. FDA also is considering whether to require that the manufacturer document the method that it used to analyze for fat and explain the reason for choosing that method. The agency believes that this information is important because the method used to analyze the excreted fat must be appropriate for the specific type of fat in the formula.

FDA also is considering whether circumstances exist that would justify establishing an exemption from the requirements to measure fat balance. FDA has tentatively concluded that the reasons and justification for such an exemption are essentially those set forth above in the discussion of proposed § 106.97(b)(2). FDA requests comment on whether, if the agency adopts a quality factor for fat, it should provide for exemptions from testing, to show that the formula meets that quality factor, such as those set forth in proposed § 106.97(b)(2), and to allow manufacturers to assure the agency that their products meet that quality factor requirement without subjecting infants to unnecessary testing.

b. *Calcium and phosphorus balance.* If FDA were to establish a quality factor for calcium and phosphorus, manufacturers would be required to collect and maintain data from clinical studies conducted in infants to show that the calcium and phosphorus contained in the infant formula are sufficient to meet the infant's requirements. There are currently no satisfactory clinical laboratory measurements that are practical for directly assessing calcium and phosphorus nutritional status in infants (Ref. 79). Furthermore, there are no accurate indirect measurements that could be made on the infant formula itself that would be useful in predicting how effective the amount and the sources of calcium and phosphorus in the formula would be in meeting the needs of infants consuming that formula. Therefore, FDA is considering requiring that manufacturers implement the recommendations of the CON/AAP Task Force and make a measurement that provides a reasonable estimate of the amount of calcium and phosphorus that is capable of being absorbed and retained for use by infants (i.e., calcium and phosphorus balance) from the formula.

FDA asks for comment concerning the appropriateness and usefulness of a measurement of calcium and phosphorus balance as one that reflects both the bioavailability of the calcium and phosphorus in the formula and how well the diet meets the metabolic requirements for these two minerals. As discussed above with regard to the conduct of trials to measure apparent fat absorption, FDA requests comment on whether it is necessary to require that a cross-over study design be used for clinical studies to measure calcium and phosphorus balance.

FDA also requests comment on what would be an appropriate duration for studies to measure calcium and phosphorus balance. The CON/AAP task force suggested that calcium and phosphorus balance studies be conducted for a 72-hour balance period after an 11-day adaptation period. FDA requests comment on whether these time periods are appropriate, both to minimize the effects of previous dietary intake on the availability of calcium from the formula being tested (Ref. 6) and to ensure that the results of the balance study are reliable and interpretable, and on whether they provide a meaningful basis on which to determine that a formula meets the quality factor requirement for calcium and phosphorus.

FDA is considering requiring that the formula used as the control in any clinical studies to measure calcium and phosphorus balance contain approximately the same calcium and phosphorus levels as the test formula because the absolute amounts of these nutrients absorbed and retained by infants may be different between formulas with different calcium and phosphorus levels. FDA is asking for comment on requirements for appropriate control formulas for calcium and phosphorus balance studies.

Amounts of calcium and phosphorus in urine and feces, along with calculated amounts absorbed and retained expressed in milligrams per kilogram and as percentages of intake, provide evidence of the rates of absorption and retention of these nutrients but do not specifically measure the ability of the formula to provide adequate calcium and phosphorus for proper bone mineralization, the most important need for these minerals in the infant. FDA is considering requiring that serum alkaline phosphatase be measured in situations in which calcium and phosphorus balance studies are required in order to assess the adequacy of formula minerals to support normal bone mineralization. Alkaline phosphatase is an enzyme involved in bone remodeling and in maintaining serum calcium concentration (Ref. 64). Increased serum alkaline phosphatase activity may be a marker of reduced bone mineralization (Ref. 80) and therefore may be useful in determining whether a formula meets a quality factor requirement for calcium and phosphorus.

Because of the limits of metabolic balance studies, including short duration, dependence on previous diet, and expense, the agency is considering the appropriateness of alternative methods for the assessment of bone mineral accretion. The agency is aware that sophisticated instruments, such as single-photon absorptiometry and dual-energy x-ray absorptiometry, have been tested for measuring bone mineral content in infants (Refs. 81 through 84), and that some authorities recommend them for determining bone mineralization in infants (Ref. 85). These types of measurements have the potential to provide an accurate measure of bone mineral accretion over the duration of use of the formula, while at the same time reducing many sources of variation inherent in balance studies. The agency is concerned, however, that these methods have not been adequately validated in infants, and that reference standards for mineralization in infants have not been established to support a requirement for manufacturers to measure bone mineralization in order to provide assurance that a formula satisfies a quality factor requirement for calcium and phosphorus. The agency asks for comment on the usefulness of these methods of analysis of bone mineral accretion in infants, and on whether they should be used in lieu of calcium and phosphorus balance studies as measurements of whether an infant formula meets the quality factor requirements for calcium and phosphorus assuming that the agency adopts such a quality factor. The agency also asks for comment on the criteria that it should use, on a case-by-case basis, in deciding whether to require these types of measures when there is particular reason to be concerned that calcium and phosphorus bioavailability may be problematic.

FDA also is considering whether circumstances exist that would justify establishing an exemption from a requirement to measure calcium and phosphorus balance. FDA has tentatively concluded that the reasons and justification for such an exemption are essentially those set forth above in the discussion of proposed § 106.97(b)(2), and requests comment on whether, if it adopts a quality factor for calcium and phosphorus, it should provide for exemptions from testing to show that the formula meets the quality