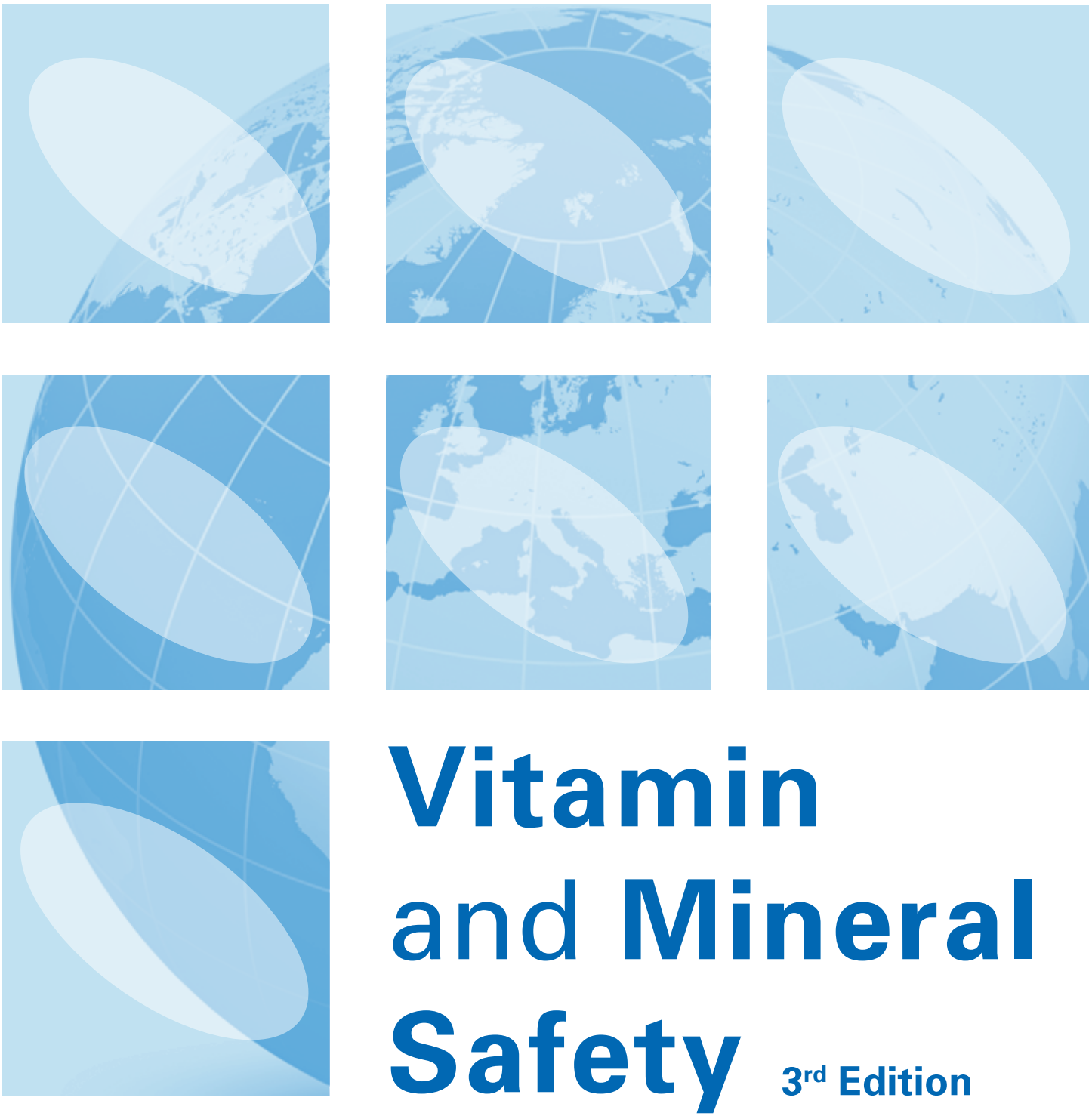

Vitamin and Mineral Safety 3rd Edition

by John N. Hathcock, Ph.D.
with a foreword by James C. Griffiths, Ph.D.

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The Science Behind the Supplements®

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Foreword

In this age of concern for not getting enough essential nutrients, i.e., vitamins and minerals, one might ask what is the utility in providing guidance on the safe upper levels? Other than calories, which many members of Western societies are over-ingesting at toxic levels, morbidity and mortality data do not seem to indicate an epidemic of vitamin and mineral toxicities. In fact, for most people, even in well-fed societies, the greater concern is nutrient deficiency. However, the popularity and usefulness of the previous two editions of "*Vitamin and Mineral Safety"* handbook, authored by John N. Hathcock, Ph.D., for the Council for Responsible Nutrition (CRN) seem to corroborate the need for scientifically-based information on how much is too much. The CRN "upper levels for supplements (ULS)," as well as the key studies and dose levels that are used to derive these values, continue to be requested and cited by scientific organizations and regulators alike. Further, the assumptions, models and data being used by regulatory authorities, including the U.S. Institute of Medicine (IOM) Food and Nutrition Board (FNB) "tolerable upper intake level (UL)," the European Commission Scientific Committee on Food (EC SCF) UL and the U.K. Expert Group on Vitamins and Minerals (UK EVM) "guidance level (GL)," make the current edition of this handbook a continued trusted resource in the international arena.

The argument has been made that Western populations may be over-consuming vitamins and minerals, since much of our basic foodstuffs are fortified, often at levels that equal or approach the current "recommended dietary allowances (RDAs)" or "nutrient reference values (NRVs)." When one adds to that one or more of the plethora of designer or tailored products appealing to the sports-minded, the sensitive demographic, the consumer picking up the latest trend, or the impulse point-of-sale promotion, then the levels of vitamin and mineral consumption may reach levels equal to some of the higher tolerable levels of nutrients that are proposed for specific sub-populations. Addition of a multi-vitamin/mineral on top of this background ingestion does lend credence to the need to have some reliable, nutrient-appropriate, scientific risk assessments for safety.

When one talks of vitamin and mineral toxicity, it is important to note that from a safety perspective there exist "tolerable upper limits" (ULs) which, after a thorough review of the underlying safety information, attempt to put a numerical value on the maximum amount a person could consume without negative effects, based solely on good science. RDAs and NRVs attempt to delineate the amount of a specific nutrient a person should consume at a minimum to derive the expected benefit afforded by such a nutrient, i.e., just enough…but not too much.

Because of the variability associated with the continuum of human consumers, i.e., intakes that are affected by gender, age, idiosyncratic sensitivities, physiological compromises, diets, and a multitude of other mitigating factors (nutritional as well as lifestyle), recommendations for normal intakes are complex and not a one-size-fits-all. This inability to provide the exact quantitative knowledge of what individuals should eat in order to maintain health makes this handbook's approach to providing guidance on the upper levels a plausible and useful reference. It is up to national, and in the case of Codex, multi-national, regulatory and standard-setting bodies to evaluate the safety data, and the efficacy data, and to set daily intake recommendations in line with the needs of their populations.

This current edition, the 3rd, has the same previously evaluated fourteen vitamins, four minerals, and ten trace elements, albeit re-examined with the addition of appropriate new references as needed. The Introduction describes the CRN Safety Methodology, the Nutrient-Appropriate Scientific Risk-Assessments, the IOM FNB Tolerable Upper Limit Method, the EC SCF UL Values and Proposal for Setting Maximal Amounts of Vitamins and Minerals in Supplements, the UK EVM Risk Assessments, and the overall CRN Approach to Supplement Safety—and these have remained intact from the 2nd edition, as has the comparison of scientifically-based risk assessment methodologies versus the RDA-based upper limit approach. In most cases, there is a wide range of safe intakes between the RDAs (or NRVs) and Upper Levels, giving consumers the ability to achieve levels within these ranges without concern for safety risks. This new edition, currently being updated chapter by chapter, provides updated research and calculations where appropriate, but continues to demonstrate that the question of over-nutrification is a very different analysis than one identifying levels of deficiency.

Methodology

Introduction

Vitamins and minerals are essential for life and health. Supplemental intakes of several nutrients provide clearly established benefits for many people, most obviously for those in specific age and gender groups. Dietary supplements are commonly used in pursuit of these benefits, as well as to provide "nutritional insurance" to those who do not know whether they are consuming recommended amounts of vitamins and minerals. For those consuming supplements for health benefits, it is important to have information about the upper levels of these nutrients that may be safely consumed.

Risk assessment is the accepted approach to evaluate the safety of any substance. The methodologies for risk assessment have been in development for decades and are accepted by the U.S.'s Institute of Medicine (IOM), the European Food Safety Authority (EFSA), and many other authoritative institutions and organizations as well as a large number of national governments.

The first step in risk assessment is to decide which type of data and what sources are relevant to the assessment. For vitamins and minerals, data from both animals and humans are available. In each of these datasets, the most reliable type of data is for overt clinical endpoints rather than for surrogate biochemical markers. Each data source has advantages and disadvantages. Animal data have the advantage of quite extensive and robust datasets and the disadvantage of requiring very uncertain and problematic extrapolation for application to humans. Human data are in many ways the exact opposite—the disadvantage is that types and amounts of human data are quite limited for many nutrients, but the advantage is that little or no extrapolation is needed for decisions that are relevant for humans.

In general, risk assessments for noncarcinogens can be separated into two main types: (1) those that depend on threshold dose-response concepts (the threshold approach) and (2) those that construct probability estimates (the benchmark dose approach). Carcinogenicity is usually treated as a nonthreshold event and for the most part will not be further considered in this document. Risk assessments that use the no observed adverse effect level (NOAEL) or the lowest observed adverse effect level (LOAEL) identify intakes that are either below (NOAEL) or just above (LOAEL) the threshold for adverse effects. The quantitative methodology for risk assessment for unknown effects of nutrients with no observed adverse effects at any intake will be discussed later in this chapter.

Studies of vitamin and mineral safety based on animal data generally use the threshold approach. In contrast, studies of drug, pesticide, and environmental chemical safety often use the benchmark dose (BMD) approach to identify an intake that produces adverse effects in some specified percentage (often 10 percent) of a population. This method constructs a probability basis for evaluating the safety of the substance being tested, but it requires an extensive database that involves administration of (or exposure to) a range of levels of the test substance at least up to those that produce adverse effects in 10 percent of the population. Such data are almost never available for human subjects, and it would be unethical to perform the experiments to obtain such data.

This third edition of the *Vitamin and Mineral Safety* handbook is based almost exclusively on human data. This methodology is based on the premise, supported by observation, that no matter how robust and extensive an animal dataset may be, the extrapolation to humans carries a very large uncertainty. This document flows from that decision and relies almost entirely on studies using human data only.

During the last several decades, the tolerable upper intake level (UL) has become internationally accepted as the best approach for nutrient safety evaluations. To this end, several international organizations and numerous government agencies have developed or accepted recommendations on UL values. These UL values may be expressed in terms of total dietary intake, supplementary amounts, or both. The UL values have been accepted by the Codex Alimentarius Commission (a food standards organization jointly formed by the UN and the World Health Organization) venture as the only valid basis for regulatory maximum limits on the contents of vitamins and minerals included in supplement products, and the adoption of this approach by Codex is leading many national governments to do likewise. The UL assumes a threshold for adverse effects and is calculated from either a NOAEL or LOAEL.

The Institute of Medicine (IOM), the European Commission's Scientific Committee on Food (EC SCF) and its successor EFSA, the UK's Expert Group on Vitamins and Minerals (EVM), industry groups, and peer-reviewed publications have all reviewed and published risk assessments for one or several of the vitamins and minerals (including trace elements). Regulatory strategies to specify maximums or other guidelines for vitamins and minerals in supplements have been or are being considered by the governments of several countries as well as by the EC, the Association of South East Asian Nations (ASEAN), and the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU). The Codex guidelines are recognized by the World Trade Organization (WTO) as being the most authoritative view on vitamin and mineral safety

and therefore have special implications for international trade. The Codex guideline is a method, approved by the Codex Alimentarius Commission, but no quantitative values have yet been identified.

The UL method as defined and implemented by the IOM is an extension of the earlier quantitative methods used in risk assessment for other substances, such as food additives and environmental chemicals. Because of the authoritative character of the IOM publications, the UL risk assessment method for nutrients has gathered widespread support and adoption by other organizations such as the EC SCF/EFSA and the EVM, with some slight modifications. All current UL methods emphasize the concept of nutrient-appropriate, quantitative risk assessment, but disparities in the selection and interpretation of available scientific literature on safety and the approach to handling uncertainty have led to sometimes large differences in the values for various nutrients. The safety evaluation method used in this document utilizes the basic features of these methods but emphasizes the direct evaluation of supplemental intakes, rather than total intakes, when feasible.

Intake Level: Definitions and Applications

There are several types of intake levels used in the literature on vitamin and mineral safety. This section reviews the definitions and differences between two of the most common ones.

Tolerable Upper Intake Level (UL)

In its report on nutrient risk assessment, the IOM states the following:

> "The *Tolerable Upper Intake Level* (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population."

For most nutrients, the UL is defined to apply to the total nutrient intake from all sources, including food, fortified foods, water, and supplements. For a few nutrients, the UL values identified apply only to supplemental sources. For example, the UL for magnesium is based on the amount needed to cause diarrhea or unacceptably loose bowels. This effect is most likely when the magnesium is consumed in a single bolus dose, and therefore the UL applies only to magnesium consumed as supplemental to the normal diet. In addition, bolus dose magnesium compounds are sometimes used as a nonprescription drug (laxative), and the UL could be used as an indicator of the dose likely needed to begin to achieve this effect.

The IOM's interpretation of this definition led them to not establish UL values for nutrients with no established adverse effects, such as vitamin B_1, vitamin B_2, vitamin B_{12}, biotin, pantothenic acid, and trivalent chromium. This interpretation was based on the premise that risk communication would adequately explain why there was no UL value for some nutrients; therefore, in these examples, a UL value would not be useful. Similar decisions have been made by EC SCF/EFSA.

Without defining the procedure or giving it a name, the EVM established guidance levels (GLs) for nutrients without sufficient evidence of adverse effects to establish a safe upper level (SUL), their approximate equivalent to the UL. The EVM expressed less confidence in the numerical values described as GLs compared with those characterized as SUL (equivalent to UL) values. Nonetheless, the interpretation and proposed use of the GL were the same as for the SUL (UL).

A major issue in setting an UL value is determining the size of the uncertainty factor (UF), or safety factor, to apply to the NOAEL or LOAEL. In some instances, credence is given to any hint whatsoever that a risk might occur at an intake below the recognized NOAEL. This point is illustrated in considerable detail in the 2010 IOM publication on vitamin D, as will be described in the Vitamin D chapter of this document.

Highest Observed Intake (HOI) Level

The highest observed intake (HOI) level was established by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) to supply guidelines for those nutrients for which there is no established UL by the IOM or SCF/EFSA. The FAO and WHO adopted the highest observed intake (HOI) under this guideline and definition:

> "The *Highest Observed Intake* is derived only when no adverse health effects have been identified. It is the highest level of intake observed or administered as reported within (a) study(ies) of acceptable quality."

Under these guidelines, a complete risk assessment for nutrients will include the identification of UL values for those nutrients with observed adverse effects and HOI values for those with no known adverse effects. The procedures for UL and HOI are identical—both are risk assessments. If evaluation of the data shows risk, a UL can be set; if no risk is found in the data analysis, an HOI can be identified as the highest intake

level with adequate data to establish that adverse effects do not occur at intakes up to that level.

In the previous edition of this document, a concept equivalent to the HOI was termed the observed safe level (OSL). This term was suggested to the FAO/WHO committee but they ultimately adopted the HOI terminology. The basic concepts of the HOI and OSL are identical. Due to the sanction of the HOI term by the FAO/WHO and further adoption in the Codex guideline on nutrient risk analysis (Codex Alimentarius Commission 2010), CRN will use the HOI term in this document.

Sources of Nutrients

To assess the safety of a nutrient, all significant sources of intake must be considered, but this is more important for some nutrients than for others. The relative importance of each source depends on several factors, including the difference between the UL value and either the recommended intake, such as the recommended dietary allowance (RDA), or the typical intakes from commonly consumed foods, as well as the chronic or acute nature of the adverse effect that is the basis of the UL.

For example, an intake equal to the UL for calcium is difficult but can be reached with consistent consumption of multiple servings (but not a single serving) of dairy foods. Moreover, excessive intakes of calcium for short periods do not lead to any acute toxicity, and acute high intakes are not known to lead to chronic adverse effects. Therefore, intakes of calcium that temporarily exceed the UL probably do no harm. In addition, calcium is a *macro*-mineral needed in relatively large amounts (approximately 1 g per day), and therefore a supplement that exceeded the UL (2,000 mg) would be noticeably large (bulky). In safety evaluations of dietary calcium, all sources should be considered: foods, fortified foods, and dietary supplements. In addition, the consumer should be aware that some nonprescription antacids have calcium carbonate as their active ingredient, but these are not known to cause any harm with short-term use.

In contrast to calcium, the UL for vitamin A (preformed vitamin A as retinol or one of its esters) can be exceeded by consistent intakes of liver or other organ meats. Furthermore, retinol is a *micro*-nutrient and the UL is only 3 mg; thus, an excessive intake could be contained in a physically small tablet or capsule. An additional factor is that adverse effects of vitamin A can be chronic (e.g., birth defects after a pregnant woman consumes far too much at a critical early stage). In safety evaluations of vitamin A, all sources of retinol must be considered, but vitamin A activity from high intakes of carotenes seems

not to produce adverse levels of vitamin A. The safety of beta-carotene itself will be discussed separately from vitamin A.

Vitamin B_{12} has no known adverse effects, and the RDA and intakes from foods are in the low microgram range. In contrast, the proposed HOI is 2,000 μg. The HOI value could be considered to apply to supplements only, with food supplying amounts that are trivial in a safety evaluation.

Methodologies for Determining Safety Levels: A Comparison

The methodology described in this document and the quantitative values identified are intended to assist in interpreting reports of adverse effects, making a quantitative approach to nutrient safety issues, and establishing policies that will help ensure consumer safety without inappropriate and unneeded restrictions based on current concepts of "nutrient need" or the composition of the most common foods. These scientific concepts and analyses are valid in any country or population with a few adjustments, such as those for nutrient intake levels related to specific local or national dietary composition and patterns. The quantitative values identified for most nutrients have sufficient margins of safety that few adjustments should be necessary. Specifically, the UL definition includes the phrase "almost all individuals in the general population" and therefore it should be valid to apply the UL values to populations with large differences in average body weight.

Some governments and agencies base their safety recommendations on the RDA of the vitamins and minerals under consideration. The sections below review the limitations of using RDA for supplements and the appropriateness of the risk assessment approach.

The Limitations of RDA-Based Methods

Recently some governments have used the RDA to set upper limits for vitamins and minerals in supplement products and have applied drug regulations on products with amounts of nutrients higher than the RDA. Although the RDA may appear to be a convenient marker, there are several problems with using the RDA in this way.

First, RDA-based limits have no scientific validity for identifying supplement safety. The RDA is not defined or identified to describe safety or represent a safety limit for either total or supplemental intake. Risk assessment is the only scientifically valid approach toward identifying supplement maximums. The Codex Alimentarius Commission, for example, has declared that maximums for nutrients in foods offered into international trade must be based primarily on risk assessment.

Second, the application of RDA-based limits to supplements leads to inconsistency in allowable ULs among products. Some countries have applied drug regulations on products with amounts of nutrients higher than the RDA. These regulations are much more stringent than regulations on conventional foods, some of which also contain many multiples of the RDA of certain vitamins. For example, the natural amounts of vitamin B_{12} in conventional foods such as liver and some shellfish can approach 100 μg per 100 g serving. The adult RDA for this vitamin is commonly set at approximately 2 to 2.5 μg. Thus, these ordinary, conventional foods may contain upward of 40 to 50 multiples of the RDA of vitamin B_{12}. Since there is no known toxicity of oral vitamin B_{12} in humans, RDA-based upper limits serve no useful purpose.

Third, RDA values, or equivalent values such as the population reference intake (PRI), are set on a very similar basis from one country to another, as they represent the consensus of scientific opinion on the nutrient quantities necessary to assure the performance of recognized and essential physiological functions. Thus, the RDA values are geared toward avoiding classical nutrient deficiency signs and symptoms or meeting some nutrient storage level deemed acceptable. Although this approach may be appropriate for helping undernourished populations identify and reach minimum levels, the data and methodology used to establish RDAs or their equivalents is not applicable to establishing safe upper levels.

Fourth, RDA-based limits are not possible for nutrients without established RDA values. For example, no RDA has been set for lutein, lycopene, boron, and many other important substances with nutritive value. These substances have beneficial effects, but the available evidence has not been judged appropriate to identify the RDA. Again, risk assessment is the appropriate methodology to identify safety limits for these important nutrients.

Fifth, drug-based regulations are not appropriate for food items for which Codex Alimentarius has established a standard or guideline. Codex is recognized by the WTO as presumptive international authority on food issues, and WTO agreements require that applied regulatory measures be no more restrictive than necessary to protect the health of consumers. The existence of a Codex guideline is direct evidence that drug-based regulations would be more restrictive than necessary.

Finally, arbitrary limits at or near the RDA may preclude certain benefits of some nutrients. There are currently many documented benefits of nutrient quantities above the RDA. For example, in 2010 the IOM updated the vitamin D RDA, which was based

entirely on the skeletal effects. Although there is strong evidence to support several other beneficial effects of this vitamin, the IOM judged the evidence insufficient to serve as the basis for an RDA value. Several of the other functions, such as neuromuscular activities, require greater amounts of vitamin D than needed for the skeletal effects. Therefore, an upper limit based on the RDA might preclude these additional potential benefits. Likewise folic acid, vitamin B_6, and vitamin B_{12} are known to help control plasma homocysteine concentrations. Homocysteine is not yet accepted as a recognized risk factor for heart disease, but there is an ever-increasing body of scientific evidence to support this conclusion. Supplementation with these three vitamins helps to control plasma concentrations of homocysteine and is likely to prove to reduce the risk of heart disease.

All of these facts point to the inappropriateness of using RDA-based limits for supplements. Labeling, not limits, can address proper usage by providing information on contents in the package, noting any benefits related to the RDA or any other measure of benefit and drawing attention to limits imposed on a safety basis, as identified by risk assessment.

The Risk Assessment Method

As indicated earlier, risk assessment is the accepted approach to evaluate the safety of substances. The methodologies for risk assessment are well established and are accepted by the IOM, EFSA, and many other authoritative institutions and organizations. The sections below address some of the recent developments and variations in risk assessment methodologies.

Nutrient Appropriateness. An important refinement of the risk assessment method is the concept of nutrient-appropriate methods. Before the advent and widespread adoption of the UL, the term *nutrient-appropriate* was used to describe risk assessment for vitamins and minerals. This terminology indicated that not all risk assessment methods are appropriate for the task. Certain risk assessment methods use default UF (sometimes called safety factors) that, although generally considered acceptable for identifying safe intakes of food additives and environmental contaminants, are unacceptably large for application in risk assessment of vitamins and minerals. Application of these factors can lead to identification of "safety limits" that are below the recommended intakes of some nutrients for certain age-gender groups. For example, the acceptable daily intake (ADI) and the reference dose (RfD) used by the U.S. Environmental Protection Agency (EPA) involve arbitrary UFs that calculate zinc safety limits below the RDA for some

populations. The benchmark dose is a probability estimate that has not been shown to be useful for human data on vitamins and minerals.

Hazard Identification. Hazard identification identifies a hazard related to excess consumption of a vitamin or mineral, using the guidelines and procedures described in the UL method. Hazard is preferably determined from human data, but animal data can be used when necessary. Biochemical or other indirect indicators should be judged to represent a hazard only if they are surrogate markers for pathological conditions. If no hazard can be identified, the additional steps in the UL method can be used to identify an HOI value. The criteria for causality should be applied, including the strength of the association, consistency of the association, specificity of the association, dose-response relationship, temporal relationship, biological plausibility, and overall coherence. If a nutrient has more than a single adverse effect, the hazard occurring at the lowest intake is the *critical effect* for this risk assessment to set a UL through the following steps. If no critical effect can be identified, the following steps allow identification of a HOI value. The Codex Alimentarius Commission uses the term *hazard* to refer to a chemical or physical agent even though the scientific publications they reference use the term *hazard* to mean an unacceptably adverse effect that is used as the basis of a policy standard or guideline. This difference in definitions should not cause a problem if it is recognized and taken into account.

Dose-Response Assessment. This process identifies a NOAEL, from human data if possible. Alternately, if the data are appropriate but do not support a NOAEL, a LOAEL may be established. Animal data are used only if appropriate human data are not available and also to guide the search for a hazard that might be identified in the human data. The uncertainties in the data are assessed and a numerical UF is assigned. It applies to the overall database and the specific data used to establish the NOAEL or LOAEL. Reasonable judgment must be applied to avoid a choice of UF that represents a worst possible but exceedingly unlikely case. If a LOAEL is used, the UF must be greater than unity (1.0) and should be appropriate for the conversion to a NOAEL. If the NOAEL or LOAEL is identified from animal data, an appropriate UF is assigned to the extrapolation to UL values for humans. If no adverse effects are known, these procedures can be used to identify an HOI.

Deriving the UL through Risk Assessment. The UL of a vitamin or mineral may be calculated through risk assessment in the following way:

UL = NOAEL ÷ UF (or UL = LOAEL ÷ UF).

If the HOI is based on sparse data, a similar procedure may be used to adjust for uncertainty in that value; however, if the total dataset is extensive (e.g., vitamin B_{12}), the absence of any adverse effect at any intake supports the argument that no correction for uncertainty is needed (i.e., the UF should be 1.0). For all nutrients with large datasets that include multiple clinical trials involving administration of a range of doses, the uncertainties may be addressed by arranging the data in decreasing order of intake and then selecting downward until confidence in the data is sufficient to justify the selection of a NOAEL or HOI with a UF of 1.0. The vitamin D chapter provides such an example.

European Commission Methodology

The Scientific Committee on Food (SCF) was established in 1974 to provide the European Commission with scientific advice on food safety. In 2002, this mandate was transferred to the newly created European Food Safety Authority (EFSA). EFSA provides independent scientific advice and communication on existing and emerging risks.

The SCF/EFSA has published UL values for several vitamins and minerals, using a methodology similar to that developed by the IOM and first published in 1997. The EC's Food Supplements Directive requires the identification of maximum amounts for supplements from risk assessments that at least nominally are derived from total intakes from all sources. No method for deriving the supplement maximums had been published by the EC as of the writing of this book. However, the approach specified in the directive would include the following steps.

Step 1. Step 1 comprises (1) use of the SCF/EFSA UL values identified through a UL method almost identical to the one developed by the IOM and (2) consideration of intakes from other dietary sources.

Step 2. Step 2 takes into "due account" population reference intakes (presumably the RDA or equivalent). However, no method for identifying or applying this due account had been published by the EC as of the writing of this book. Two industry associations have proposed that the RDA could be used along with the ULs and intakes from food sources to calculate a population safety index that separates the nutrients into three categories that demand different levels of regulation and monitoring.

CRN suggests that the population reference intakes referred to in step 2 could also be used to ensure that the risk assessment and identification of other intakes are not excessively conservative, thereby producing a UL and a supplement maximum below the RDA.

The EC proposal would seem to need to identify maximums for supplements plus fortified foods as the differences between the UL and the intakes from other sources. That is, the supplement maximum would be the UL minus the expected intake from conventional foods. The EC has not yet proposed how it will allot the difference between the UL and unfortified intakes into fractions for supplementation and increased fortification, or for the variations in expected intake from one country to another or from one dietary pattern to another. It has been reported that the EC is working on establishing dietary "supplement maximums" by using risk assessments done by EFSA, but has not done so for any of the nutrients. These values with corresponding analysis will be added in future editions when this information becomes available.

EVM Methodology

The risk assessments in the EVM report on vitamin and mineral safety are based on the UL method developed by the IOM, but they assigned the term *safe upper level* (SUL) to the values derived by this method. The EVM stated that for most nutrients, the databases were not sufficient to set an SUL; therefore, a guidance level (GL) was identified. Nonetheless, this GL was often derived and used for overall safety evaluation and discussion of policy options in the same manner as a SUL value. In contrast to CRN's views, the EVM used animal data to identify some SUL values entirely on the basis of high-quality animal data, despite the great uncertainty inherent in quantitative extrapolation from animals to humans.

For a few nutrients, the EVM report takes an additional step toward risk management recommendations for supplements. A safety value based on supplemental intake effects could be logically used to identify maximum contents of products marketed and regulated as supplements. Indeed, most of the SUL and GL values identified by EVM were based on supplemental intakes. In these cases, the EVM uses data on typical intakes from foods along with the supplemental SUL or GL to calculate these values for total intakes. In addition, the EVM explicitly states that it assumes daily consumption throughout the adult lifetime (age 16 years to death), whereas the IOM and SCF/EFSA are not explicit on this issue for all nutrients.

Methodologies for Determining Supplement Safety: A Summary

The premise of this handbook is that the safety evaluation for dietary supplements is best determined on a case-by-case basis through nutrient-appropriate risk assessment, and not as arbitrary multiples of the RDA. Scientific assessments used to identify adequate intake levels (RDAs or their equivalents) are not well suited to identifying hazards. Nutrient-

appropriate risk assessment incorporates internationally recognized methodology and is grounded in sound toxicological principles.

Nutrient-appropriate risk assessment requires the safety evaluation to depend on identification of a hazard causally related to excessive intake, assessment of the dose-response relationship for the identified hazard, consideration of uncertainty, and, finally, derivation of a supplementation level that is not only safe but also includes a reasonable margin of safety.

In the identification of a hazard related to excessive consumption of a nutrient, care must be taken to distinguish between effects that represent a genuine hazard and those that are merely a nuisance. For example, the minor gastrointestinal distress that can occur when supplements are taken on an empty stomach should not be considered equivalent to the risk of a serious consequence, such as liver toxicity. Similarly, the dermal "flushing" that can be produced by nicotinic acid is a definite nuisance but does not produce any known pathology. Nonetheless, the IOM, EC SCF/EFSA, and EVM used flushing as the critical effect to establish UL or equivalent values. (For more details, see the Niacin chapter.)

Direct Safety Evaluation of Supplemental Intakes

If appropriate data on supplemental intakes of a specific vitamin or mineral are available, the safety may be determined by risk assessment directly on those data, as illustrated by the EVM report. If the supplemental intake dose-response relationship is identified from the strongest data and assessed conservatively, no additional uncertainty factor is needed (that is, the implicit UF is 1.0). For some nutrients, the NOAEL or HOI data are related to the use of *supplemental amounts* of the vitamin or mineral, above and beyond the amounts contributed by the diet; therefore, such data do not require any additional consideration of amounts contributed by consumption of conventional foods.

The expected intakes of most nutrients from conventional foods do not invalidate this approach for two primary reasons: either (1) intakes are small in comparison with the UL or HOI (e.g., for vitamin B_{12}) or (2) the evidence for the safety of supplemental amounts was developed under conditions in which the amount of the nutrient consumed from conventional foods was well known (e.g., in the case of selenium). These considerations are taken into account in each section on the specific nutrients.

Indirect or Difference Method for Supplement Safety

If appropriate data on supplemental intakes of a vitamin or mineral are not available, a *difference* procedure, similar to that identified by the EC, may be used. The difference method involves the following:

- Determination of the UL or HOI for total intake from all sources.
- Identification of the usual intakes from conventional foods from appropriate food intake surveys and food composition tables, taking consumption of fortified foods into account. There is considerable controversy about the selection and management of the intake data. Which source of data is appropriate? What percentile of intake should be considered?
- Calculation of the UL for supplements as a difference. This method still leaves an unresolved dispute about how to allocate this difference between supplementation and fortification and how to account for differences in dietary patterns and composition.

Characteristics of CRN's Safety Methodology

The CRN approach as described above includes the basic elements of both the IOM and FAO/WHO methods. For some nutrients, the CRN and EVM methods are the same; for others, CRN and the EVM use different approaches. CRN's principal points of departure from all three of these approaches include the following:

- CRN gives preference to data on effects of *supplemental* intakes, rather than total intakes, thereby eliminating any need to correct for intakes from conventional foods.
- CRN gives stronger preference to use of human data over animal data, thereby avoiding the uncertainties involved in extrapolation between species.
- CRN gives stronger preference to clinical trial data from human studies, if available, but also uses epidemiologic data.
- CRN gives stronger preference to identifying NOAEL values than to LOAEL values, thereby eliminating the uncertainty related to extrapolation downward from the LOAEL.
- CRN considers only effects that represent a true hazard (i.e., risk of impaired health) rather than nuisance effects.
- CRN preferentially uses direct evidence of adverse effects, if available, rather than biochemical markers or other indirect indicators.
- CRN utilizes history of use data, if necessary, to identify an HOI and UL when adverse effects in humans have not been identified for a nutrient. This approach relies on previous human experience when consistent with the scientific evidence that for some nutrients includes an indication of a high order of safety.

- CRN conservatively selects human NOAEL values that justify selection of an UF of 1.0, thereby eliminating the need to select a specific numerical value.
- CRN recognizes that supplement use is an independent choice for the consumer and does not impose increased intake on anyone who does not select it. This contrasts with food fortification programs that require the consumer to carefully scrutinize labels in an effort to obtain or avoid increased intake of nutrients.

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Vitamin A

Introduction

Vitamin A has essential actions in areas of health that include vision, cellular differentiation, organ development during embryonic and fetal growth, and membrane structure and function. Several other complex physiological processes, including growth, reproduction, and immune system functions, also depend on vitamin A (Ross 1999).

In regard to eye health and function, the vitamin plays at least two distinct roles: (1) as a retinal-opsin complex that serves as a phototransducer and (2) in the maintenance of the various eye membranes. In the first case, deficiency of vitamin A in the retina leads to night blindness. In the second, deficiency can lead to xerophthalmia, with a loss of the basic integrity of the eye structure and possible total blindness. Vitamin A deficiency is the foremost cause of preventable blindness in the world.

Safety Considerations

Vitamin A is fat-soluble and readily accumulates in the liver. Therefore, if taken at high dosages on a daily basis, the vitamin can easily accumulate to dangerous levels in the liver and other tissues. However, in nutritionally deprived populations who do not have a steady, sufficient intake of vitamin A, the same high dosages may be necessary as occasional supplements in order to prevent the severe health consequences of vitamin A deficiency. This makes the safety of vitamin A highly dependent on both the daily level of intake and the duration of consumption. The vitamin A status of the populations under study is critical to conclusions about safety, and nutritionally replete and nutritionally deficient populations must be considered separately.

The potential for adverse effects from excessive vitamin A intake in nutritionally replete populations is well documented. Conceivable risk is based on the ingestion of large amounts of preformed vitamin A in the form of retinol or retinyl esters, but not from provitamin A varieties such as beta-carotene or other provitamin A carotenoids. There are no examples of vitamin A toxicity resulting from high intakes of beta-carotene or other carotenoids. (The safety of beta-carotene itself is a separate question and will be addressed in the section on that nutrient.) Vitamin A is listed on food and dietary supplement labels in international units (IU), while nutrition scientists commonly use mg or µg retinol activity equivalents (RAEs). The conversion rate between an IU and RAE are 1 IU retinol = 0.3 µg RAE. The following sections discuss the possible adverse effects of preformed vitamin A.

Liver Abnormalities

Because the liver is the principal storage site for excess vitamin A, a causal relationship between very high intakes and liver toxicity is well established in both animals and humans. The adverse effects can include reversibly elevated liver enzymes as well as other conditions with greater persistence, such as fibrosis, cirrhosis, and even death (Institute of Medicine [IOM] 2001). The human data, however, are often confounded by other factors such as alcohol intake, infectious hepatitis, hepatotoxic drugs, and pre-existing liver disease. Consumption of 25,000 to 50,000 IU of preformed vitamin A per day for periods of several months or more can produce multiple adverse effects, including liver toxicity (Hathcock et al. 1990); but the effects in this intake range may be dependent on compromised liver health or function. A supplemental intake of approximately 25,000 IU is the lowest dose at which such effect can be confidently attributed to vitamin A in persons with mildly or moderately compromised liver health (Geubel et al. 1991).

Birth Defects

The smallest daily supplement generally considered to generate any risk of birth defects is also 25,000 IU (Hathcock et al. 1990); this amount may be considered to be the LOAEL. A report by Rothman et al. (1995) concluded that there was a significantly increased risk of neural crest birth defects at maternal daily supplemental levels of "more than 10,000 IU," but how much more was not stated. The report findings are complicated by the fact that, although the average supplemental intake by these women was 21,675 IU, the authors did not identify individual supplemental intakes in the seven cases involving birth defects. All seven cases involved intakes at levels greater than 10,000 IU, but how much greater is not known. Several issues have been raised about the validity of the defect classification scheme used and the resulting likelihood that the study overestimated the risk associated with vitamin A at the levels identified in this study (Oakley and Erickson 1995; Shaw et al. 1996; Werler et al. 1996). The finding by Rothman et al. was not confirmed by a more recent study (Lammer et al. 1996).

A few reports suggest the possibility that there may be some risk of vitamin A toxicity at supplementation levels below 20,000 IU per day. One report to the FDA suggested a characteristic birth defect in association with maternal supplementation at 18,000 IU per day (Rosa et al. 1986). Another report found marginal indications of adverse effects on the liver in elderly subjects with chronic supplementation at levels of 5,000 to 10,000 IU per day (Krasinski et al. 1989). This observation has not been confirmed (Stauber et al. 1991), and the same laboratory was unable to repeat this finding in later research (Johnson et al. 1992). Results of a trial in nonpregnant women documented that daily oral

vitamin A supplements of 4,000, 10,000 and 30,000 IU given for 3 weeks were in the range of endogenous plasma levels of vitamin metabolites that are seen in women in early stages of pregnancy (during organogenesis). Therefore, a dose of 30,000 IU per day "should be considered as non-teratogenic in [humans]" (Wiegand et al. 1998). No other reports have indicated adverse effects from vitamin A at these supplemental levels.

Bone Fragility

Recent reports are conflicted on the issue of vitamin A's effect on bone fragility. Some have suggested that relatively low intakes of preformed vitamin A (that is, retinol and retinyl esters) could increase bone fragility and risk of hip fracture. Other studies do not support such an effect.

In January 2001, the IOM released its review of vitamin A and other micronutrients. In assessing the safety of vitamin A, the IOM considered possible adverse effects in relation to bone mineral density and hip fracture, concluding that the studies are "provocative but conflicting, and therefore they are not useful for setting a UL for vitamin A."

The evidence that the IOM reviewed relating vitamin A to potential adverse effects on bone included animal studies, human mechanistic studies, and epidemiological evidence. Animal and human biochemical data indicate a mechanism for possible adverse effects of retinol on bones, but this research does not establish the occurrence of these effects in humans consuming practical levels of vitamin A. A single-dose clinical trial by Johansson and Melhus (2001) confirms the mechanistic effect in humans—but with a single dose of over 27,000 IU of retinol (as 15 mg of retinyl palmitate). This clinical study confirms and refines previous knowledge about interactions of vitamin A and vitamin D.

At the time of the IOM review, the only epidemiological study suggesting an adverse relation between high levels of vitamin A intake and bone health was the 1998 Swedish population study by Melhus and coworkers (Melhus et al. 1998). Melhus and coworkers interpreted those data as showing a significant increase in the risk of hip fracture when retinol intakes reached 5,000 IU per day. Other epidemiological studies available at that time, including those by Freudenheim et al. (1986) and Houtkooper et al. (1995), found no such relationship with retinol intakes of up to 6,600 IU per day.

A study from the National Health and Nutrition Examination Survey (NHANES III) by Ballew et al. (2001) examined, but could not detect any relationship between plasma retinyl esters and bone density. Plasma retinyl esters are good indicators of excessive vitamin A intake, and bone density is an excellent indicator of the resistance of bones to

fracturing. The NHANES III study is a large survey of a cross-section of the entire U.S. population. A small epidemiological study in Iceland also found no relationship between vitamin A and bone density (Sigurdsson et al. 2001).

The publication of results from the Nurses' Health Study (NHS) (Feskanich et al. 2002) measured the risk of hip fracture in comparison with vitamin A intakes estimated from food frequency recalls. In addition to the recognized inherent limitations of observational research, the NHS study reported a significantly below-average rate of total hip fracture in all postmenopausal women studied; results were limited to self-reported hip fractures with no event adjudication or identification of exact fracture site. Furthermore, multiple observations of this specific population over time initially led to statistical associations of milk and calcium with increased risk of hip fracture, but these apparently erroneous implications disappeared with continued collection of data.

An observational study of serum retinol concentrations and bone fractures supports a relationship between retinol and increased health risk, especially for fractures of the hip (Michaelsson et al. 2003). The level of dietary intake associated with the increased risk is not apparent from this study. The ability of retinol to induce the resorption of bone may be ameliorated by adequate intake of vitamin D (Boucher 2003).

Vitamin A in Nutritionally Deprived Populations

In some countries where widespread, endemic vitamin A deficiency results in large-scale occurrence of health defects—especially blindness related to xerosis and xerophthalmia—and mortality. In response, current public health programs and medical practice include the administration, once every 3 to 12 months, of 50,000 to 200,000 IU or more vitamin A as retinyl esters to children for the treatment and prevention of vitamin A deficiency (International Vitamin A Consultative Group [IVACG] 1984; Ross 1999). Even though the dosage (which varies according to the age of the child) appears high, it must be noted that this is not a daily dose but rather a periodic dose, administered to a target population with depleted liver stores of vitamin A and a large unused storage capacity. Such extremely high intakes of vitamin A would not be tolerated in populations that are nutritionally replete but are essential in populations with chronic vitamin A deficiency.

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IOM (2001). Based on the available data, the IOM identified a NOAEL of 15,000 IU per day and applied a UF of 1.5 to arrive at a UL of 10,000 IU per day, based on possible

birth defects as the critical safety issue. The IOM evaluated the evidence for vitamin A increasing bone fragility and concluded that those data were insufficient to serve as the basis for a UL value.

European Commission's Scientific Committee on Food (EC SCF 2002). The EC SCF identified a UL of 10,000 IU of retinol per day. Like the IOM, the EC SCF based its recommendation on the risk of birth defects only, as evidence of increased risk of bone fragility is not compelling.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM concluded that retinol intakes of 10,000 IU per day are not teratogenic but that intakes greater than 5,000 IU per day may increase bone fragility. It found no threshold for the bone fragility effects and set 5,000 IU per day as a guidance level rather than as an SUL.

CRN Recommendations

CRN considers supplements of 10,000 IU per day of preformed retinol to be safe for most people. As stated earlier, the recommendations for nutritionally replete populations must be considered separately from nutritionally deprived populations. In addition, even within nutritionally replete populations, intake from food sources can vary widely. Therefore, for people who consume high levels of vitamin-A-fortified foods or liver, a lower limit of 5,000 IU per day is recommended.

The CRN recommendations are based on evidence of birth defects risks at higher levels. The evidence for bone fragility has been conflicted, and recent studies indicate, if anything, that the preponderance of evidence may have moved away from the suggestion that vitamin A might increase the risk of hip fracture. Therefore, bone fragility is not used as a factor in CRN's recommendations.

The CRN recommendations are based on the following considerations as well:

- The LOAEL for birth defects is at least 25,000 IU of retinol per day, and there are no credible data to suggest that it is likely to be lower than 21,675 IU per day.
- The IOM selected a retinol NOAEL of 15,000 IU per day, but conservatively applied an uncertainty factor of 1.5 to derive a UL of 10,000 IU per day.
- The intake of retinol and retinyl esters from sources other than supplements is likely to be less than 3,400 IU per day (Feskanich et al. 2002).
- There is a long history of safe use of dietary supplements containing 5,000, 8,000, and 10,000 IU per day.

- The IOM NOAEL equivalent to 15,000 IU per day and the highest likely intake of 3,400 IU per day from sources other than supplements are compatible with the CRN UL of 10,000 IU per day.
- Persons with likely high intakes of retinol (e.g., those who regularly consume liver or other organ meats) should not consume supplements that contain preformed vitamin A; they may, however, safely consume vitamin A as beta-carotene.
- Some companies limit the amount of retinol in multivitamin products to 5,000 IU per day or less to avoid possible concerns about bone fragility.

Quantitative Summary for Vitamin A

CRN UL, supplemental intake	
Low consumers of fortified foods and liver	10,000 IU (3,000 µg RAE)/day
High consumers of fortified foods and liver	5,000 IU (1,500 µg RAE)/day
IOM UL, total intake	10,000 IU (3,000 µg RAE)/day
EC SCF UL, total intake	10,000 IU (3,000 µg RAE)/day
EC supplement maximum	Not determined
EVM, guidance level, total intake	5,000 IU (1,500 µg RAE)/day

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Beta-Carotene

Introduction

Beta-carotene is one of many hundreds of food carotenoids, relatively only few of which have been studied in relation to their impact on human physiology. Beta-carotene is the most abundant form of provitamin A in fruits and vegetables (Olson 1994; Ross 1999). The other two carotenoids with vitamin A activity, alpha-carotene and beta-cryptoxanthin, are not prevalent in foods. Beta-carotene is an effective source of vitamin A in both conventional foods and vitamin supplements, and it's generally safe.

Epidemiological studies have shown that people with high intakes of beta-carotene or high blood levels of this nutrient have a reduced risk of various diseases, including cancer and heart disease (van Poppel and Goldbohm 1995). The chemical abilities of beta-carotene to quench singlet oxygen and to inhibit peroxyl free-radical reactions are well established (Sies and Stahl 1995). In addition to this antioxidant property, beta-carotene and some other carotenoids may play an important role in facilitating normal cell-to-cell communication through gap junctions (Acevedo and Bertram 1995). Because many carcinogens inhibit gap junction communications (Gregus and Klaassen 1996), protection of this activity by dietary substances could be an important function in the protection against cancer.

The suggestion that beta-carotene might reduce the risk of cancer is based on epidemiological evidence but has not been confirmed by clinical trials. The few clinical trials that have directly sought to determine whether beta-carotene supplements would reduce the risk of cancer have led to surprising results, including the suggestion that the nutrient could have a harmful effect on smokers.

The results of the Carotenoid and Retinol Efficacy Trial (CARET study) (Omenn et al. 1996) and the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC 1994) showed increased rates of lung cancer with beta-carotene supplementation in long-term smokers who continued to smoke. Conversely, the Physicians' Health Study (PHS) (Hennekens et al. 1996) found that beta-carotene had no effect, either helpful or harmful, on any cancer. The ATBC and CARET trials studied populations at very high risk for lung cancer, and because the duration of treatment was far shorter than the induction time for this cancer, these trials ultimately do not support—but also do not disprove—the hypothesis that beta-carotene could be anticarcinogenic in the early stages of cancer.

Safety Considerations

Beta-carotene has been considered virtually nontoxic because humans tolerate high dietary dosages without apparent harm (Bendich 1988; Hathcock et al. 1990; Diplock 1995). Standard toxicological tests, including teratogenic, mutagenic, and carcinogenic assays, have been performed on beta-carotene without any evidence of harmful effects. There is no evidence that conversion of beta-carotene to vitamin A contributes to vitamin A toxicity, even when beta-carotene is ingested in large amounts (Olson 1994). The only documented biological effect of high beta-carotene intake has been discoloration of the skin related to hypercarotenemia, but this occurs only at extremely high intake levels. Intakes as high as 180 mg per day have been given to humans for several months without observed adverse effects other than changes in skin color (Mathews-Roth 1986).

Because of the extensive toxicological safety record of beta-carotene, clinical trials were designed with the assumption that the only likely effects would be beneficial. However, questions about the safety of beta-carotene have been raised by the results of the ATBC and CARET trials, which observed significant increases in lung cancer risk for long-term smokers and asbestos workers who were given beta-carotene supplements of 20 or 30 mg per day. On the other hand, there was evidence in the CARET study that beta-carotene may reduce the risk of lung cancer in former smokers. In contrast to the unexpected increases in lung cancer risk in the ATBC and CARET trials, no increased risk was observed in the PHS trial, which included more than 2,000 smokers and lasted approximately 12 years, compared with the 5 to 7 years in the ATBC and CARET trials. Three other, shorter-term trials had similar results (Greenberg et al. 1990; Blot et al. 1993; Greenberg et al. 1994). Moreover, observational studies have found that a reduced risk of lung cancer and other diseases accompanies increased beta-carotene intake (Menkes et al. 1986; Rimm et al. 1993; Hennekens 1996). It has been postulated that the effects of alcohol or of high levels of retinol intake on the liver might explain the adverse outcomes with beta-carotene in the ATBC and CARET studies (Lachance 1996).

In 2012, the European Food Safety Authority (EFSA) reviewed the possible link between the ingestion of beta-carotene and cancer enhancement in heavy smokers. EFSA noted the findings of the ATBC study and CARET trials and also identified a meta-analysis of randomized controlled trials (RCT), which demonstrated a lack of protection associated with beta-carotene supplementation against cancer risk (Druesne-Pecollo et al. 2010). The meta-analysis, which included the ATBC and CARET trials, indicated an overall increased risk of lung cancers in subjects supplemented with beta-carotene compared with placebo. In subgroup analyses, increased risk of lung cancers was also reported when supplemental beta-carotene was provided in combination with other antioxidants, in individuals supplemented with 20 to 30 mg beta-carotene per day, in populations

composed only of smokers or asbestos workers, and in populations with a majority of men. In contrast, no increased lung cancer incidence was reported at supplemental dose levels of beta-carotene varying from 6 to 15 mg per day for about 5 up to 7 years. EFSA concluded that exposure to beta-carotene from its use as a food additive and as a food supplement at a level below 15 mg per day does not give rise to concerns about adverse health effects in the general population, including heavy smokers.

Studies using ferrets, which in contrast to rats and mice metabolize beta-carotene in a manner similar to that of humans, demonstrated inconsistent findings with respect to lung carcinogenesis. Results of one study suggest that high intakes of beta-carotene (in an unstable, nonprotected form) may increase the risk of lung cancer as shown by histopathological changes, especially in the presence of cigarette smoke (Liu et al. 2000; Wolf 2002); however, experiments conducted with protected forms of beta-carotene did not show histopathological changes in the lungs (Kim et al. 2006; Fuster et al. 2008). The limited animal studies are not sufficient to confirm a cancer risk in humans and also do not provide an adequate basis for a quantitative extrapolation to a safe or unsafe human intake.

A clinical trial of the impacts of beta-carotene (25 mg per day) and/or vitamins C and E (1,000 mg and 400 IU, respectively) indicated that among subjects who neither smoked nor drank alcohol, beta-carotene strongly reduced the risk of recurrent colorectal adenomas; but among smokers and drinkers, beta-carotene increased the risk (Baron et al. 2003). These data provide further evidence that beta-carotene has different effects on smokers and nonsmokers.

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Institute of Medicine (IOM 2000). The IOM found no effects of high beta-carotene other than carotenodermia, and it judged this effect to be cosmetic rather than adverse. Consequently, the IOM did not set a UL based on this effect. The organization did find that there was a potential for beta-carotene to increase the risk of lung cancer in smokers, but considered the evidence to be inconsistent and not sufficient for a dose-response assessment and the derivation of a UL value.

European Commission, Scientific Committee on Food (EC SCF 2000). The EC SCF found a possibly increased risk for smokers with beta-carotene supplementation of 20 mg or more per day, but concluded that there were insufficient data to set a precise figure for a UL. In addition, it noted that the evidence was insufficient to evaluate the safety of different isomeric forms in different preparations.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM considered the evidence of increased cancer risk in smokers consuming 20 mg of beta-carotene per day to be compelling but of uncertain application to other persons. Thus, it identified a LOAEL of 20 mg and cautiously selected a UF of 3 to derive an SUL of 7 mg for most adults. Furthermore, it recommended that smokers or those exposed to asbestos refrain from taking any supplemental beta-carotene.

EFSA (2012). EFSA concluded that beta-carotene exposure at a level below 15 mg per day, from its use as a food additive and as a food supplement, does not give rise to concerns with regard to adverse health effects in the general population. It also stated that no sensitive groups were identified from the available evidence at this exposure; therefore, the term *general population* encompasses all groups, including heavy smokers.

CRN Recommendations

Extensive data show that beta-carotene supplements of 50 mg every other day (the equivalent to 25 mg per day) can be taken for more than a decade without harm in a large group of mostly nonsmokers (Hennekens et al. 1996). An intake of 25 mg per day is therefore selected as the highest observed intake (HOI) for nonsmokers. Skin discoloration may occur with larger amounts, but this effect should be considered undesirable rather than adverse. It is harmless and self-correcting with intake reduction.

The only evidence of adverse effects of beta-carotene comes from the ATBC and CARET studies, which involved long-term heavy smokers and asbestos workers. These data suggest a LOAEL of 20 mg per day for smokers or asbestos workers, but disparities between the ATBC and CARET results and other data prevent confident identification of any LOAEL for beta-carotene. Smokers and asbestos workers should first control these health risks, then evaluate whether beta-carotene supplements are safe.

Quantitative Summary for Beta-Carotene

CRN UL, supplemental intake	25 mg/day for nonsmokers; smokers should not use
IOM UL, total intake	Not determined
EC SCF UL, total intake	Not determined
EFSA, food additive and supplement maximum	<15 mg/day
EC supplement maximum	Not determined
EVM SUL, supplemental intake	7 mg/day for nonsmokers; smokers should not use

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Vitamin D

Introduction

Vitamin D is required in quantities smaller than any other fat-soluble vitamin. The major forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D has been described as a pro-hormone or sunshine-dependent vitamin. Some dietary vitamin D_2 comes from plants, but the largest contribution to dietary intake of vitamin D is the vitamin D_3 in fish liver oils, eggs, milk, and liver. Milk is commonly fortified with 10 µg (400 IU) of vitamin D_3 per quart. Extremely high potency (40,000 to 50,000 IU) products—sold as prescription or sometimes over the counter—often consist of vitamin D_2. Most vitamin D dietary supplements contain vitamin D_3. The conversion of international units to metric weights is extremely simple for both vitamin D_2 and D_3: 1 µg equals 40 IU; 0.025 µg equals 1 IU.

Vitamin D_3 (or D_2) from endogenous production, foods, or vitamin supplements is inert and must undergo two hydroxylation reactions in the body for activation. The first occurs in the liver and the second in the kidneys. The first hydroxylation produces 25-hydroxy vitamin D (25[OH] D) and the second produces the active hormone 1,25-dihydroxy vitamin D (1,25-di[OH]D). Because the plasma half-life of the active form 1,25-di(OH)D is short (approximately 15 hours), the best indicator of vitamin D status is 25-(OH)D (plasma half-life of 15 days or so). Because of these pharmacokinetics, most vitamin D in plasma is present as 25(OH)D, also called calcidiol, and its concentration has become the standard index of vitamin D status. 1,25-di(OH)D (calcitriol), the activated vitamin, regulates intestinal absorption and plasma concentration of calcium. As calcitriol, vitamin D is fundamentally involved in the formation of bone, and so its deficiency can lead to rickets in children or osteoporotic changes in adults.

Bioavailability

Although it can be synthesized in the body with sufficient exposure to sunlight or another ultraviolet (UV) light source, most people are not exposed to such UV light in consistent and sufficient quantities. No extra vitamin D is required when skin exposure to UV light is ample; but without such exposure, a person is completely dependent on ingested vitamin D. The UV component of sunlight converts internally produced 7-dehydrocholesterol naturally present in the skin to cholecalciferol (vitamin D_3) (Life Sciences Research Office [LSRO] 1978; Holick 1999).

Although adequate UV light exposure can provide sufficient vitamin D, many elderly persons have limited sunlight exposure, inadequate dietary sources, and a decreased ability to activate vitamin D, making them susceptible to vitamin D deficiency (Gloth et al. 1995; Holick 1999). Elderly people are likely to have substantially increased needs for dietary vitamin D because of their decreased mobility and exposure to sun and decreased activation in the liver and kidneys.

Thus, the nutritional need for dietary vitamin D depends on the biosynthesis in the skin, which in turn is influenced by time of exposure to sunlight, season (sun intensity and clothing), latitude, skin pigmentation, and the use of sunscreens.

Safety Considerations

The formation of vitamin D in the skin is slowed once dietary vitamin D intakes are sufficient and blood levels of the activated forms are high. Therefore, excess exposure to sunlight does not lead to vitamin D toxicity (Holick 1999; Hathcock et al. 2007).

Dietary vitamin D can, however, produce toxic effects when consumed in very large quantities, especially over an extended period of time. Studies have shown that subjects with abnormally high levels of vitamin D intake can suffer from a wide range of signs and symptoms, from dehydration to permanent mineral deposits in soft tissues, including muscle, heart, kidney, and cartilage. Continued intake of toxic levels can have severe and persistent adverse consequences. The widespread occurrence of vitamin D overdoses in British children just after World War II, caused by consumption of excessively fortified milk, led health professionals to be extremely conservative in estimating safe levels for vitamin D.

Prolonged intake of excess vitamin D may lead to predictable increases in plasma 25-hydroxy vitamin D concentrations (Institute of Medicine [IOM] 2011), and the increase is directly proportional to the vitamin D dose (Barger-Lux et al. 1998). Treatment with vitamin D or 25-hydroxy vitamin D does not generally increase the serum concentrations of the active metabolite (calcitriol, 1-alpha,25-dihydroxycholecalciferol, or 1,25-dihydroxy vitamin D) (Barger-Lux et al. 1998). Nonetheless, excess vitamin D can have toxic effects, perhaps because of the increases in blood concentrations of 25-hydroxy vitamin D, a form that can overstimulate intestinal absorption of calcium and cause excessive calcium mobilization from bone and hypercalcemia (Norman 1996; Holick 1999). Although the IOM considers hypercalcemia to be the critical effect in vitamin D toxicity, no clinical trials have used levels sufficiently high to produce such high blood

levels of calcium. All such evidence comes from reports of anecdotal cases of accidental massive overdoses.

The amount of daily vitamin D ingestion needed to produce adverse effects varies widely. In most adults, daily intake in excess of 50,000 IU (1.25 mg) is needed to produce toxicity (Miller and Hayes 1982). Clinical trials in the last decade or so have found no hypercalcemia in subjects in subject taking 10,000 IU (250 µg) in long-term clinical trials. In certain disease conditions such as sarcoidosis, mycobacterium infections such as tuberculosis, or idiopathic hypercalcemia, toxicity can occur at levels of vitamin D intake lower than those in healthy persons. A causal relationship between excess vitamin D intake and hypercalcemia is unlikely, although people with idiopathic hypercalcemia may be subject to adverse effects of vitamin D at lower intakes than are comfortably tolerated by healthy individuals (Expert Group on Vitamins and Minerals [EVM] 2003).

Body size matters. One study has found that in children of unspecified body weight (probably between 10 and 30 kg), the amount of dietary vitamin D causing adverse effects may be as low as 2,000 to 4,000 IU (50 to 100 µg) per day. In full-term infants, adverse effects are reported to occur with intakes as low as 1,800 IU (45 µg) per day (Chesney 1989), but no adverse effects occurred in a 6-month study of infants given 1,600 IU per day (Fomon et al. 1966). Numerous reports confirm a variety of adverse effects at very high intakes when vitamin D is used as a drug, whether administered parenterally or in activated forms (Nanji 1985; Goldman and Wheeler 1987; Schwartzman and Franck 1987; Allen and Shah 1992; Boulard et al. 1994; Oymak et al. 1994; Matsukawa et al. 1995). These circumstances do not relate to the usual oral intakes of vitamin D from foods or dietary supplements, and such reports provide no useful information about the safety of dietary sources of vitamin D.

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Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM did not find the data sufficiently compelling to identify a NOAEL or a LOAEL. Instead, it set a guidance level of 25 µg, based on studies by Vieth et al. (2001) at 100 µg and Johnson et al. (1980) at 50 µg. The EVM concluded that long-term use of supplements of 25 µg is "well tolerated," but did not establish a safe upper limit.

IOM (2011). The IOM has established a UL of 100 µg (4,000 IU) vitamin D, based on downward extrapolation from the NOAEL of 250 µg (10,000 IU), as derived from multiple clinical trials in a risk assessment using UL methodology (Hathcock et al. 2007). To take into account uncertainties from emerging data regarding all-cause mortality, chronic disease risk, and falls at serum 25(OH)D levels of approximately 75 to 125 nmol

per L, the IOM considered the findings of Heaney et al. (2003) in establishing a UL. In this study, intakes of 5,000 IU vitamin D per day for 160 days resulted in serum 25(OH)D levels that did not exceed 150 nmol per L and serum calcium levels within normal ranges. The UL was set at 20 percent below 5,000 IU (i.e.¸4,000 IU) because of the uncertainties surrounding the data and the reliance on one study. The IOM intended "not to determine that certain levels of intake definitively cause harm, but rather to decide whether the emerging data were sufficiently compelling to warrant caution relative to vitamin D intakes."

The IOM approach, however, did not take into account the ample caution built into the NOAEL and the UL method in the peer-reviewed literature (Hathcock et al. 2007). In addition, the IOM examined the data related to several endpoints but based their UL of 4,000 IU on only one of several different adjustments of the data on all-cause mortality in several different age, gender, and disease groups (Visser et al. 2006).

For example, Figure 6-1 of the IOM report graphs the hazard ratio of all-cause mortality against serum 25(OH)D (nmol per L), with adjustments for various health and age factors. All risk curves are high when vitamin D status is low and progress downward to low points as vitamin D status increases. Although the two right side points are equal, there is no hint of a reverse J-shape to the curves—and thus no indication that that vitamin D status up to 82 nmol per L causes any harm.

Figure 6-2 plots relative risk of death in elderly people against baseline serum 25(OH)D, graphed with four different adjustments for factors that could influence health. Only Model 4 shows definite increases in risk as serum 25(OH)D reaches 75+ nmol per L. This model adjusts the data for gender, age, education, chronic disease, serum creatinine concentration, and lifestyle variables including smoking status, alcohol consumption, and physical activity; it adjusts for all these plus frailty indications including physical activity, low serum albumin concentration, and low serum total cholesterol concentration.

Figure 6-3 displays the results of adjustment to the data similar to that in Figure 6-2's Model 4, and there is no increase in risk with serum 25(OH)D concentrations up to 80.3+. There was no discussion of the likelihood of a random "significant" effect when more than 20 adjustments are made to the primary data.

EFSA (2012). EFSA considered that a daily vitamin D dose of 250 µg per day reflects a NOAEL, based on two studies in which doses of 234 to 275 µg vitamin D_3 per day for 8 weeks to approximately 5 months did not result in hypercalcemia in healthy young men (Barger-Lux et al. 1998; Heaney et al. 2003). A UF of 2.5 was applied to account for the

variation in the sensitivity of the population to potential adverse effects of long-term vitamin D exposure, the short duration of the studies, as well as the small number and characteristics (healthy young men with minimal sun exposure) of the individuals studied. The UL was therefore estimated to be 100 µg per day.

CRN Recommendations

The traditional—but not data-based—conservatism of vitamin D recommendations is rapidly being corrected to evidence-based assessments. These assessments indicate that larger amounts are now considered safe for most persons.

The data by Heaney and coworkers (2003) indicate that the NOAEL for vitamin D is at least 250 µg (10,000 IU). Thus, from the available data, the LOAEL is greater than 250 µg per day in relation to its hypercalcemic effects. The IOM and EVM estimate vitamin D intakes from all nonsupplement sources to be in the range of 360 IU (9 µg) or less. Many dietary supplements that include vitamin D contain 10 µg (labeled in the U.S. as 400 IU) or less, although some recent formulations contain 600 to 800 IU. There are no reports of adverse effects at these levels of intake. It is noteworthy that hypercalcemia has never been observed in a causal relationship to vitamin D in a randomized clinical trial. All the evidence for vitamin D causing hypercalcemia comes from anecdotal reports of accidental or misinformed consumption of much higher amounts.

With recent clinical trial data in mind, the CRN UL for supplements is identified as 250 µg (10,000 IU), based on the absence of adverse effects at this level of supplementation in clinical trials of sufficient size and duration and under a variety of conditions (Hathcock et al. 2007; IOM 2011). Having confidence in the safety of the 250 µg intake level, CRN does not consider this NOAEL µg to need adjustment by application of a UF (i.e., a UF of 1.0 is applied). Thus, CRN sets the UL for supplements at 250 µg (10,000 IU).

Quantitative Summary for Vitamin D

CRN UL, supplemental intake	250 µg (10,000 IU)/day
IOM UL, total intake	100 µg (4,000 IU)/day
EFSA UL, total intake	100 µg (4,000 IU)/day
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	25 µg (1,000 IU)/day

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Vitamin E

Introduction

Vitamin E is a complex substance that comes in eight forms: alpha-tocopherol, beta-tocopherol, gamma-tocopherol, delta-tocopherol, and the esters of each. Alpha-tocopherol ester is the most common form used in manufactured foods and supplements, while gamma-tocopherol is the most common form in the natural food supply (though the most common form can vary by geographic region) (Traber 2006). It is undetermined whether there are differences in health benefits among these different forms. There are equal numbers of a closely related group of vitamins called tocotrienols. Their chemical forms (alpha, beta, gamma, and delta, and their esters) are closely analogous (but not identical) with tocopherols, but tocotrienols are not included in this chapter because there is comparatively little research available on them.

Vitamin E's only known role is that of an antioxidant and a scavenger of free radicals, making it effective as a protector of the integrity of lipids and phospholipid membranes. Unlike other vitamins, vitamin E has not yet been shown to be directly associated with the function of any enzyme system (Sokol 1996). As an antioxidant, vitamin E is strongly interactive with other dietary systemic antioxidants such as vitamin C and glutathione and several enzyme systems such as glutathione reductase and superoxide dismutase. The most common test for vitamin E deficiency is the hemolysis of erythrocytes in vitro under the influence of hydrogen peroxide. In test systems commonly used alpha-tocopherol is the most potent, but the amounts of the gamma form far exceed the alpha forms in oils, such as soybean oil. Therefore, their relative importance as dietary antioxidants is uncertain (Institute of Medicine [IOM] 2000; Traber and Manor 2012).

Vitamin E has been shown to be essential to human health in several ways. One of the earliest observations of the physiological effects of vitamin E deficiency relates to reproduction. In deficiency models in female animals, their fetuses died and were resorbed; in males, the testes became atrophied. Indeed, the chemical name for vitamin E, tocopherol, is related to this protective effect on reproduction (Nelson 1980). In addition, numerous scientific reports, which include mechanistic data (Sies and Stahl 1995), epidemiology (Knekt et al. 1991), and some human intervention clinical trials, support the hypothesis that vitamin E is associated with a decreased risk of heart disease and certain cancer. Clinical trials overall, however, have produced mixed results on whether vitamin E protects against heart disease and cancer. In summary, vitamin E is clearly

essential and may reduce the risk of some chronic diseases, and it has a wide margin of safety (Bendich and Machlin 1988; Dickinson 2002; Higdon 2004).

Safety Considerations

The scientific literature contains many reports of safe continuous intake of vitamin E supplements at levels that are many multiples of the current RDA. The evidence comes from different types of studies, ranging from observational studies of a few subjects to large randomized, controlled intervention trials looking for effects on cancer, cardiovascular disease, and other disorders. There have been dozens of published studies with documented safety observations for vitamin E supplements, involving a total of more than 100,000 people.

Gillilan et al. (1977). In a double-blind crossover study by Gillilan and his colleagues, 48 patients with stable angina documented by electrocardiography and angiography were randomly assigned to receive vitamin E at 1,600 IU per day for 6 months, either before or after a 2-month placebo period. Although vitamin E did not appear to improve the symptoms or exercise capacity of these patients with well-established heart disease, it did prove entirely safe. The patients showed no differences in symptomatic or laboratory indices of heart disease between the active therapy and placebo periods.

Meydani et al. (1998). Meydani and her colleagues conducted an extensive 4-month safety study of vitamin E at 60, 200, or 800 IU per day in 88 healthy elderly persons. None of the subjects reported any side effects, nor did they show any abnormalities on a wide array of laboratory tests that studied plasma proteins and lipids; glucose; lipoproteins; bilirubin and other parameters of liver, kidney, and metabolic function; red blood cell counts; bleeding time and other parameters of coagulation; and a wide range of immune function indicators.

Cambridge Heart Antioxidant Study (CHAOS) (Stephens et al. 1996). The safety findings from the relatively small trials by Gillilan et al. and Meydani et al. were corroborated by the larger Cambridge Heart Antioxidant Study (CHAOS), in which 2,002 patients were randomized to receive a placebo or vitamin E at 400 or 800 IU per day. Over a median follow-up of 510 days, no significant adverse effects of vitamin E supplementation were reported among these patients with symptomatic and angiographic coronary disease. Indeed, the rate of treatment discontinuation stemming from adverse effects—a common gauge of patient tolerance—was only 0.55 percent for the entire population, with no difference between the actively treated and control patients.

Heart Outcomes Prevention Evaluation Study (HOPE Study Investigators 2000). The Heart Outcomes Prevention Evaluation (HOPE) study was an evaluation of the angiotensin-converting enzyme (ACE) inhibitor ramipril and/or 400 IU per day of vitamin E per day in 9,541 patients with multiple cardiovascular risk factors. According to the HOPE investigators, "Vitamin E was well tolerated, with no significant adverse events as compared with placebo" over the mean follow-up of 4.5 years. Note that this interpretation by the authors did not stop the decisive inclusion of one narrow segment of these data in a meta-analysis of vitamin E intake and all-cause mortality (Miller et al. 2005), even though the "significant" endpoints were 1 of 23. The usual 5 percent probability threshold for significance is 1 of 20, so the 1 of 23 is well within the normal range of expectation based on random effects.

Roche European American Cataract Trial (REACT) (Chylack et al. 2002). Nor was vitamin E safety an issue in the Roche European American Cataract Trial (REACT), in which 297 patients with age-related cataracts were randomized to receive a placebo or an antioxidant cocktail containing vitamin E at 600 mg per day along with vitamin C and beta-carotene, a nutrient that is a biochemical precursor to vitamin A. In this trial, 78 percent of the patients were followed for 2 years, 53 percent for 3 years, and 12 percent for 4 years.

Age-Related Eye Disease Study (AREDS) (Age-Related Eye Disease Study Research Group 2001). The 3,640 patients with vision loss or eye lesions who were being seen at retinal diseases clinics in the Age-Related Eye Disease Study (AREDS) were also randomized into placebo or antioxidant-cocktail groups; additionally, zinc supplementation was compared with a placebo. The patients took the cocktail—which contained 400 IU vitamin E as well as vitamin C and beta carotene—daily for a mean of 6.3 years. The AREDS researchers singled out a significant increase in skin yellowing—a classic sign of high beta-carotene intake—as the only notable apparent side effect of antioxidant therapy.

Brown and Colleagues (Brown et al. 2001; Cheung et al. 2001). Brown and coworkers tested the combination of simvastatin and niacin, with or without an antioxidant cocktail containing vitamin E at 800 IU per day, against either the cocktail alone or matching placebos in 160 patients with clinical coronary disease, low levels of high-density-lipoprotein (HDL) cholesterol, and normal levels of low-density-lipoprotein (LDL) cholesterol. No adverse effects were observed in patients who received antioxidants alone, but there was an unexpected blunting of the favorable HDL-elevating response to simvastatin-niacin in those who received antioxidants plus the drug treatments.

DATATOP (Parkinson Study Group 1998). The DATATOP clinical trial, which followed on 800 subjects for 8.2 years, found no adverse effects of 2000 IU of vitamin E per day. This study supports the safety of very high intakes of vitamin E over a long period.

Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study (ATBC 1994). Against this backdrop of multiple observational and prospective, randomized trials suggesting excellent safety for vitamin E supplementation stands the ATBC Cancer Prevention Study, which raised a flag of caution. Among 29,133 male smokers in Finland, ages 50 to 69 years, vitamin E ingested at 50 mg per day for 5 to 8 years was associated with a 7.8 percent rate of death from hemorrhagic stroke, compared with a 5.2 percent rate for the placebo (66 cases in the vitamin group, compared with 44 in the controls). The authors did not discuss the nearly significant decrease in occlusive stroke, a much larger group than those with hemorrhagic stroke. Overall, there was a nearly significant decrease in total strokes. As with many antioxidants, care must be exercised when exogenous factors are already compromising the health status, which, besides the smoking mentioned above, also includes concomitant use of pharmaceuticals (Hemilä and Kaprio 2011; Rutkoswki and Grzegorczyk 2012).

In the ATBC study vitamin E was also associated with a lower incidence of prostate cancer and reduced mortality from ischemic stroke and ischemic heart disease. But no degree of statistical significance was provided for any of these apparent differences. The authors concluded only that the observation of a higher hemorrhagic-stroke mortality with vitamin E "requires careful review."

Such careful review has occurred; in a further evaluation, these same researchers concluded that "alpha-tocopherol supplementation increases the risk of fatal hemorrhagic strokes but prevents cerebral infarction" (Leppälä et al. 2000). In this study, within 3 months of the initial stroke diagnosis there were 85 deaths from subarachnoid hemorrhagic stroke, with the group supplemented by vitamin E seeing 28 more such deaths, or 50 percent more, than the control group. By contrast, the 807 deaths from cerebral infarction suffered by those with vitamin E supplementation represented 53 fewer deaths, or a decrease of 14 percent, when compared with the group that was not taking vitamin E supplements. The overall net effects of vitamin E on incidence of and mortality from strokes were statistically nonsignificant, but the numbers of total stroke deaths were actually lower with vitamin E treatment.

The literature contains a few reports, in addition to that of the ATBC trial, that tentatively associate bleeding complications with vitamin E supplementation. Such reports

sometimes involve persons with vitamin K deficiency, especially in conjunction with chronic anticoagulant therapy such as warfarin (Coumadin) or high-dose aspirin. These associations have led some reviewers to recommend caution and observation in patients on taking both vitamin E supplements and long-term warfarin (Spencer 2000).

It has been suggested that high intake of vitamin E may influence coagulation in some persons with vitamin K deficiency, but not in those persons with adequate vitamin K levels—in other words, the overwhelming majority of the population (Corrigan and Ulfers 1981; Corrigan 1982; Kappus and Diplock 1992; Dowd and Zeng 1995). Indeed, a recent large trial of patients on long-term warfarin who also took 800 to 1,200 mg of vitamin E showed no changes in coagulation parameters that would suggest an increased bleeding risk (Kim and White 1996).

The findings of the ATBC trial have not altered the prevailing consensus that vitamin E intake up to the UL is safe. The IOM report that delineated the dietary reference intake (DRI) values for vitamins E and C concluded that the "preliminary" ATBC findings were "not convincing" in the absence of corroboration in other large-scale clinical trials (IOM 2000).

When stated in a misleading way, some comments can become self-fulfilling prophesies. For example, one paper is titled "no evidence supports vitamin E indiscriminate supplementation" (Dolan et al. 2009). At face value, this statement is true, but it is equally true for all substances. With the word "indiscriminate" included, the statement logically applies to everything: food, supplements, and drugs.

Meta-Analyses Studies. Meta-analysis is the combination of data from multiple studies to increase the total number of subjects being considered—that is, to increase the replication and statistical power, thus enabling the detection of small differences. At first glance, this would seem to be a great advance—that is, to increase the statistical power to detect small but real effects. And this would be true if two critical factors are appropriately considered and accounted for: (1) the statistical (mathematical) formulas must be appropriate and valid, and (2) the inclusion/exclusion criteria must be appropriate. Unfortunately, both aspects are commonly abused.

A prime example is the meta-analysis of high-dosage vitamin E supplementation reported by Miller and coworkers (2005). In this publication, the authors graphed the intake of vitamin E against the risk of "all-cause mortality." Lower intakes provided risk below the zero line (suggesting a protective effect) and at 400 IU or higher the risk line moved just above zero (suggesting adverse effects). For some unexplained reason, the authors

changed the mathematical formula from a linear equation at low risk to a quadratic equation when the risk line was positive (above zero). Apparently, this was the only method that gave a statistically significant indication of harm by vitamin E. Even with this mathematical manipulation, the results indicated harm by vitamin E only if the WAVE trial (Women's Angiographic Vitamin and Estrogen Trial; Waters et al. 2002) was included—it indicated by far the highest risk of adverse effects by vitamin E in any trial. The meta-analysis authors made no note of the letters to the editor that pointed out the shortcomings of the WAVE study—those researchers measured 22 different parameters and found 1 to be statistically elevated, and they made no mention of their 5 percent probability definition of "statistically significant." Thus, one would expect 1 of 20 parameters to be elevated on a random basis (1 of 20 is 5 percent). Without the WAVE trial data, meta-analysis would have shown nothing—even with the questionable statistical manipulations. In this example, the authors violated the principles of both critical criteria: (1) invalid statistical methods, and (2) inclusion regardless of the shortcomings of the clinical trial. Note that, in the WAVE study, the "significant" effects were 1 of 23 endpoints whereas 1 of 20 would be expected on a random basis.

Moreover, the meta-analysis authors used a fixed effects model, rather than the generally more valid random effects model to calculate the effects. Considering these and other statistical issues, prominent statisticians have reexamined the data considered in the meta-analysis, and more recent data, and concluded that "vitamin E intake is unlikely to affect mortality regardless of dose" (Berry et al. 2009).

In another meta-analysis example, the statistical procedures were apparently valid, but the inclusion/exclusion criteria were not presented in a logical fashion (Bjelakovic et al. 2007). In this meta-analyses of mortality in randomized trials, the researchers included beta-carotene, vitamin A, vitamin C, vitamin E, and selenium because they are all "antioxidants." This makes no more sense than including both glucose and cyanide data in the same meta-analysis because both are "carbon compounds." More specifically in this meta-analysis, selenium is known to decrease the risk of cancer in selenium-deficient high-risk groups in China, and beta-carotene is known to increase the risk of cancer in smokers and asbestos workers. Thus, including a known negative and known positive in a single meta-analysis should be expected to result in a null effect because the two effects cancel each other. This procedure amounts to erasing data that show meaningful effects. In this example, the statistical methods seem to have been acceptable but the inclusion/exclusion criteria were simply invalid.

A recent publication by the Cochrane Summaries (Bjelakovic et al. 2012) selected the same authors of the previously discussed meta-analysis (Bjelakovic et al. 2007) to

perform an "independent" review. The meta-analysis authors have a clearly established negative view of antioxidants, and it is mystifying how the Cochrane Summaries could consider their review to be independent. The Cochrane Summaries reached almost exactly the same conclusions as the previously cited meta-analysis (Bjelakovic et al. 2007). The selection of these reviewers raises concerns that the Cochrane managers had a viewpoint in advance of consideration of the scientific data.

Official Reviews

IOM (2000). The IOM reviewed all data relevant to vitamin E safety but did not identify a human NOAEL or LOAEL. Instead, it identified a LOAEL of 500 mg per kg per day from animal data and calculated a human UL by applying a composite UF of 36. Assuming a body weight of 68.5 kg and rounding off the value, the calculated UL is 1,000 mg per day for adults. Although the different chemical forms of vitamin E have different potencies (that is, IU per mg) for beneficial effects, the IOM concluded that potency for potential adverse effects is not known to vary in an analogous manner, and therefore the IOM did not differentiate between *all-rac* and *RRR* alpha-tocopherol with regard to possible adverse effects. Hence, the IOM applied a uniform UL value to all forms of vitamin E.

European Commission, Scientific Committee on Food (EC SCF 2003). The EC SCF reviewed all the evidence and found no adverse effects for oral vitamin E in humans. Declaring the evidence at higher intakes to be insufficient, the EC SCF selected the clinical study by Meydani and colleagues (1998) to identify a NOAEL of 800 IU per day, or approximately 540 mg per day. Judging the database to be only moderately robust, the EC SCF applied a UF of 2, converting from IU to mg to derive a UL of 270 mg per day, rounded up to 300 mg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM identified an SUL range of 800 to 1600 IU based on the Meydani and Gillilan studies (Gillilan et al. 1977; Meydani et al. 1998), and then used the more conservative value of 800 IU value to calculate a vitamin E SUL of 540 mg per day.

CRN Recommendations

To simplify safety considerations of different forms of vitamin E and yet reach appropriately cautious conclusions, CRN recommends conversion of the IU to mg alpha-tocopherol equivalents (alpha-TE). Because most clinical trials have been conducted with

synthetic *dl*-alpha-tocopheryl acetate (that is, *all rac*-alpha-tocopheryl acetate in the currently accepted scientific nomenclature), conversion of a UL for supplements in IU to the corresponding vitamin E activity in mg alpha-TE will result in a more conservative UL. CRN identifies a vitamin E UL of 1,600 IU from clinical trial data that showed no adverse effects at this level of intake (Gillilan et al. 1977). Correspondingly, CRN considers 1,600 IU as the upper limit to have a very low level of uncertainty because of the absence of adverse effects at the higher intake of 3,200 IU (Anderson and Reid 1974). With the conversion to mg alpha-TE as performed by the EVM, the CRN upper limit for supplements of 1,600 IU is equivalent to 1,073 mg, a value very similar to that identified by the IOM through extrapolation from animal data. The CRN upper limit for supplements applies to healthy adults who are not taking any anticoagulant drug.

Quantitative Summary for Vitamin E

CRN UL, supplemental intake	1,000 mg (1600 IU)/day
IOM UL, total intake	1,000 mg/day
EC SCF UL, total intake	300 mg/day
EC supplement maximum	Not determined
EVM SUL, supplemental intake	540 mg (800 IU)/day

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Vitamin K

Introduction

Vitamin K is a group of fat-soluble vitamins that are essential for biosynthesis of proteins involved in blood coagulation and metabolic pathways in bone and other tissues. This group includes two natural vitamers: vitamins K_1 (phylloquinone) and K_2 (menaquinone) (Institute of Medicine [IOM] 2001). Bacteria in the colon can convert vitamin K_1 to K_2. In addition, bacterial enzymes typically lengthen the isoprenoid side chain of vitamin K_2 to produce a range of K_2 forms. Three synthetic types of vitamin K are known: vitamins K_3, K_4, and K_5. Although the natural K_1 and all K_2 homologs have proven to be nontoxic, the synthetic K_3 (menadione), K_4, and K_5 have shown toxicity.

Vitamin K is effective in treating deficiency produced by coumarin-based drugs, such as warfarin (Coumadin) and certain other anticoagulants. The Food and Drug Administration (FDA) has not approved any form of vitamin K for the prevention or treatment osteoporosis (Kanai et al. 1997); however, vitamin K_4 has been shown to decrease the fracture rate in animals up to 87 percent. In the amount of 4 mg daily, vitamin K has been approved by the Ministry of Health in Japan since 1995 for the prevention of osteoporosis (Feskanich et al. 1999; Iwamoto et al. 1999).

Safety Considerations

No toxicity has been observed with high doses of the two natural forms of vitamin K: vitamin K_1 and vitamin K_2. Hence, no UL for these two forms has been established (IOM 2001; Expert Group on Vitamins and Minerals [EVM] 2003; Rasmussen et al. 2005).

Blood clotting studies in humans using 45 mg per day of vitamin K_2 (Ushiroyama et al. 2002) and even up to 135 mg per day (45 mg three times daily) showed no increase blood clot risk (Asakura et al. 2001).

Unlike the natural forms of vitamin K, however, the various synthetic isomers—K_3 (menadione), K_4, and K_5—are demonstrably toxic. Large doses have been shown to cause allergic reactions, hemolytic anemia, and cytotoxicity in liver cells (Higdon 2004). The FDA has banned all synthetic vitamin K products for over-the-counter sale in the U.S.

Official Reviews

IOM (2001). The IOM found no reports of adverse effects for vitamin K_1; hence, it

concluded that there was no basis for a LOAEL or a NOAEL value. Lacking a LOAEL or a NOAEL, no UL value was established.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM also cited Craciun et al. (1998) as evidence of a lack of adverse effect at 10 mg of K_1 per day. Because of the small size of that trial, the EVM selected a UF of 10 to correct for potential interindividual variation and therefore calculated a guidance level of 1 mg per day.

European Food Safety Authority (EFSA 2006). EFSA recognized that no adverse effects occurred in a small, short-term clinical trial of 10 mg of K_1 per day (Craciun et al. 1998). Given these findings, EFSA did not set a UL value.

CRN Recommendations

Vitamin K in its natural forms has an extremely low potential for toxicity, but the data are insufficient to establish just how low. The EVM's application of a UF of 10 seems unnecessarily cautious in view of the absence of reports of adverse effects at intakes of 30 mg or more, although data to support the 30 mg value are sparse. Consequently, CRN identifies the UL for vitamin K as 10 mg per day. This value is based on the same clinical data identified by the EVM (Craciun et al. 1998) but without the tenfold UF. Dietary intake and intestinal biosynthesis are trivial in comparison with the UL of 10 mg. Because of the strong interaction of vitamin K with anticoagulant drugs, the UL does not apply to individuals taking such medications.

Quantitative Summary for Vitamin K

CRN UL, supplemental intake	10 mg/day
IOM UL, total intake	Not determined
EFSA UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	1 mg/day

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Vitamin C

Introduction

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. It is required for the synthesis of collagen, which is a critically important structural component of blood vessels, tendons, ligaments, and bone. Also, vitamin C is necessary for the synthesis of carnitine, a small molecule that is essential for the transport of fat into cellular organelles such as mitochondria, where the majority of fat oxidation occurs.

Vitamin C is a highly effective antioxidant, especially in the acidic environment of the stomach. In this way, it may be important in blocking production of compounds such as nitrosamines, most of which are known carcinogens (Tricker and Preussmann 1991). This action is consistent with the lower rates of stomach cancer in persons with high vitamin C intakes (Scanlan 2000).

Unlike most mammals and other animals, humans do not have the ability to make their own vitamin C. Therefore it must be obtained from the diet (Higdon 2004). The intestinal absorption of vitamin C is regulated by at least one dose-dependent active transporter (National Institutes of Health [NIH] 2013). Cells accumulate vitamin C via a second specific transport mechanism. In vitro studies show that oxidized vitamin C (dehydroascorbic acid) enters cells via some facilitated glucose transport and is then reduced internally to ascorbic acid. The physiological importance of oxidized vitamin C uptake is unknown.

Overall vitamin C concentrations in the tissues and plasma are tightly controlled. At oral intakes above 1 g per day, vitamin C uptake falls off to less than 50 percent, and unmetabolized vitamin C is excreted in the urine (NIH 2013). It does not necessarily follow that unabsorbed vitamin C provides no benefit; for example, decreases in nitrosamine production may be beneficial in reducing stomach cancer risk.

Safety Considerations

Vitamin C has very low toxicity and is not believed to cause serious adverse effects at high intakes. The most common complaints after very high intakes of vitamin C are diarrhea, nausea, abdominal cramps, and other gastrointestinal effects related to the osmotic effect of unabsorbed vitamin C in the intestine (Institute of Medicine [IOM] 2000).

Although large intakes of ascorbic acid may cause transient gastroenteritis or diarrhea in some individuals, several more serious adverse effects have been purported in books and review articles. These effects have not been supported by the evidence in regard to oral consumption (Hathcock and Rader 1990; IOM 2000), but fears about them continue to persist. The following sections review the main hypothesized adverse effects and the evidence surrounding them.

Conditioned Scurvy

This spurious phenomenon has been so widely cited in review articles that it has become unsupported "conventional wisdom." It is said to result from a conditioning of adults who have had large intakes of vitamin C and in infants whose mothers consumed large amounts during pregnancy, such that blood levels are rapidly depleted to scorbutic levels after discontinuation of ascorbic acid.

Detailed review, including bibliographic tracing, does not substantiate this phenomenon (IOM 2000). High intakes result in accelerated clearance, but this does not result in blood levels lower than normal and nowhere near scorbutic levels (Schrauzer and Rhead 1973; Tsao and Leung 1988). A paper commonly cited in support of conditioned scurvy in infants whose mothers took vitamin C was speculative and did not provide data that supported such a relationship (Cochrane 1965). Oral scurvy due to withdrawal from high vitamin C intakes was reported in another paper (Siegel et al. 1982), but the diagnosis was not confirmed, the time to onset was suspiciously short, and no plasma vitamin C determinations were made. In summary, conditioned scurvy has not been substantiated, despite very large numbers of people taking vitamin C quantities of 1 g or more over the last 40 years.

Oxalate Kidney Stones

Early reports of large increases in urinary oxalate levels following high intakes of vitamin C speculated that oxalate production increased with high intakes of ascorbic acid in an analytical procedure that involved heat (Hoffer 1985). More recent reports based on better assay procedures have indicated a small but significant increase in oxalate excretion (10 to 15 mg, still within the normal range) by persons consuming 1,000 mg of ascorbic acid daily (Levine et al. 1996), though this result might be caused by the instability of ascorbic acid in the urine during collection, storage, or analysis. Some reports assert that ascorbic acid is a risk factor for calcium oxalate kidney stones (Urivetzky et al. 1992). Other research involving alternative sample handling procedures found no increase with a different preparation of ascorbic acid at intakes of up to 8 g per day (Fituri et al. 1983). One study found that oxalate production occurred only in the

urine sample in vitro with oral ascorbic acid intakes of up to 10 g (Wandzilak et al. 1994).

A significant contribution of high ascorbic acid intakes to urinary oxalate is not established (Costello 1993), and the association of oxalate kidney stones with higher ascorbic acid intakes remains speculative (Gerster 1986). Indeed, the available epidemiological evidence suggests the exact opposite: a decreased risk of oxalate kidney stones with increased intake of vitamin C. For example, a prospective epidemiological study found the relative risk of oxalate renal stones to be decreased for men consuming 1,500 mg or more vitamin C in comparison with those consuming less than 250 mg (Curhan et al. 1996). These data provide further support for an earlier retrospective study (Fellstrom et al. 1989) that produced similar results. An authoritative review found no risk of oxalate kidney stones in relation to vitamin C intake (IOM 2000).

Increased Uric Acid Excretion

Similar to the increased oxalate concern, it has been theorized that a large increase in urate excretion could increase the risk of urate renal stones. For example, a significant increase in uric acid excretion has been reported with vitamin C intakes of 1,000 mg and higher (Levine et al. 1996), and a single dose of 4 g ascorbic acid has been reported to increase fractional clearance of uric acid (Stein et al. 1976). Five other studies, however, show no effect on uric acid excretion of vitamin C intakes of up to 12 g per day (IOM 2000). The clinical effects of the increased uric acid production, if any, have not been identified.

Pro-Oxidant Effects, Excessive Iron Absorption, and Excessive Iron Release

A potential for harm from high intakes of ascorbic acid through pro-oxidant effects has been widely discussed (Herbert 1993; Herbert 1994; Herbert et al. 1996), but an authoritative review discredited such claims (IOM 2000). Some research (Kondo et al. 1988) has been cited (Herbert et al. 1996) as demonstrating that an ascorbate-driven free radical reaction damages cells. This research, which used in vitro studies with phagocytes, found increased release of iron from senescent erythrocytes by the phagocytes only at abnormally high ascorbic acid concentrations. The concentrations used were more than tenfold above the highest plasma ascorbic acid levels of subjects consuming 1,000 to 2,500 mg of ascorbic acid per day (Levine et al. 1996). The hypothesis that high intakes of ascorbic acid will produce direct pro-oxidant effects is not consistent with the data on iron release and contrasts with the antioxidant effects of vitamin C observed under a wide variety of conditions (Frei 1991).

The concept that the enhancement of iron absorption by ascorbic acid leads to excess iron-related disease has also been suggested (Herbert et al. 1996) based on the iron-heart disease hypothesis (Sullivan 1981; Salonen et al. 1992). This hypothesis—that high iron status produces an increased risk of heart disease—is not supported by subsequent evidence and evaluation (Aronow 1993; Baer et al. 1994; Liao et al. 1994; Morrison et al. 1994; Moore et al. 1995; Sempos et al. 1996). Furthermore, ascorbic acid intakes of 2,000 mg per day for 2 years did not cause excessive iron uptake (Cook et al. 1994). And intakes of up to 10,000 mg per day for up to 3 years have been observed in clinical trials without side effects (Bendich and Langseth 1995). These findings provide additional evidence that high ascorbic acid intake is unlikely to produce any iron-related increase in heart disease. Moreover, endogenous ascorbate prevented, rather than promoted, lipid peroxidation in iron-overloaded plasma (Berger et al. 1997).

Vitamin B_{12} Destruction

The in vitro observation of the apparent destruction of vitamin B_{12} by ascorbic acid (Herbert and Jacob 1974) has been erroneously interpreted as an adverse effect of vitamin C. Vitamin C intakes of up to 4 g per day have no effect on vitamin B_{12} status (Afroz et al. 1975; Ekvall et al. 1981). A major review found no evidence that vitamin B_{12} antagonism is a credible adverse effect of vitamin C (IOM 2000).

Erosion of Dental Enamel

Chewable vitamin C tablets, used daily, have been reported to lead to erosion of dental enamel because of the acidity of ascorbic acid (pH of 2.8) and the abrasiveness of the tablets (Guinta 1983). However, this result occurs only if the tablets have not been properly formulated to a pH of approximately 4 to 5 using sodium ascorbate or another buffering agent. Chewable vitamin C supplements that are properly buffered do not cause dental enamel erosion.

Gastrointestinal Distress

The only concretely documented adverse effects of high vitamin C intakes are gastrointestinal symptoms such as nausea, abdominal cramps, and diarrhea of osmotic origin (Miller and Hayes 1982). When these effects occur, the vitamin C dosage is usually 3,000 mg per day or higher, taken at one time; but a few individuals respond at single doses as low as 1,000 mg (IOM 2000). This effect results from a direct osmotic effect of unabsorbed ascorbic acid and can usually be avoided by taking the vitamin as a buffered salt rather than as a free acid. The symptoms usually disappear within a week or two with no further consequences.

Official Reviews

IOM (2000). The IOM found no credible reports of adverse effects other than gastrointestinal distress related to irritation and osmotic diarrhea from large doses. For these effects, the IOM identified a LOAEL of 3,000 mg per day but, because of the mild and transient nature of the effects, selected a UF of 1.5, thus deriving a UL of 2,000 mg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM also found no credible reports of adverse effects other than mild gastrointestinal distress and diarrhea. The EVM applied a standard toxicological default UF of 3, setting a guidance level of 1,000 mg per day.

European Food Safety Authority (EFSA 2004). In April 2004, EFSA released a risk assessment for ascorbic acid that included some evidence of oxidative damage. Their report noted that genotoxicity was supported by oxidative damage that could occur in the presence of transition metal ion. However, EFSA theorized that such effects were countermanded by the antimutagenic effects in a variety of systems. Vitamin C would be expected to be antimutagenic because of its antioxidant properties, and there are several types of data consistent with this effect. EFSA concluded that the data are insufficient to establish a UL for vitamin C. It was noted, however, that doses of vitamin C up to about 1 g in addition to normal dietary intakes are not associated with adverse gastrointestinal effects, but that such effects may occur at higher intakes (3 to 4 g per day). There are no data on the gastrointestinal absorption or tolerability of esterified forms of vitamin C.

CRN Recommendations

Vitamin C has an extremely low potential for toxicity. Multigram supplements have been widely used for decades, with only mild and transient gastrointestinal effects such as irritation, bloating, and diarrhea. The adverse gastrointestinal effects of very high intakes justify the establishment of a UL at 2,000 mg per day.

CRN also identified a LOAEL of approximately 3,000 mg. Given the mild, transient, and self-correcting nature of the adverse effects, CRN considers an uncertainty factor of 1.5, as identified by the IOM, to be ample.

The IOM and the EVM set their ULs at 1,000 mg per day, but neither considered in detail the role of individual dosages versus total intake. CRN recommends a UL of 2,000 mg

per day but spread out into at least two doses. Single doses should not exceed 1,000 mg in order to avoid undesirable gastrointestinal effects.

Quantitative Summary for Vitamin C

CRN UL, supplemental intake	2,000 mg/day
IOM UL, total intake	2,000 mg/day
EFSA UL, total intake	Not determined
EC, supplement maximum	Not determined
EVM, guidance level, supplemental intake	1,000 mg/day

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Vitamin B_1 (Thiamin)

Introduction

Thiamin is a sulfur-containing member of the water-soluble B-complex family, which is essential for normal development, growth, reproduction, lactation, and physical performance. It is involved in releasing energy from macronutrients that provide energy, especially from carbohydrates. Thiamin is an essential vitamin that cannot be synthesized and must come from the diet. Thiamin is widely distributed in small amounts in foods, but it is easily lost during the milling, heating, canning, blanching, and storage of foods. It is readily absorbed from the intestine and readily excreted through the kidneys (Tanphaichitr 1999; Expert Group on Vitamins and Minerals [EVM] 2003).

Thiamin is especially sensitive to the antinutritive effects of excess alcohol consumption (Tanphaichitr 1999), which decreases the absorption of thiamin and increases its excretion. Alcohol also inhibits the activation of thiamin to its coenzyme forms. Overt thiamin deficiency in Western countries occurs mostly among alcoholics. Thiamin deficiency may result from dependence on unfortified, polished rice as the staple food and from the consumption of a diet that is limiting in other respects.

Safety Considerations

Oral thiamin, or vitamin B_1, is virtually nontoxic, as demonstrated by a long history of use as an oral supplement—often as many multiples of recommended intakes—without adverse effects. In fact, there are no reports of adverse effects of oral thiamin, even at dosages of several hundred milligrams (Life Sciences Research Office [LSRO] 1978; Department of Health, Education, and Welfare [DHEW] 1979; Institute of Medicine [IOM] 1998). Rare cases of allergic sensitivity are documented and have occurred solely in patients who received thiamin by the parenteral route (Miller and Hayes 1982; Wrenn et al. 1989). These reactions have no apparent relevance to the safety of oral intake and may have been related to the injection vehicle. The efficiency of thiamin absorption rapidly declines when intake reaches 5 mg. This limitation has been cited as a possible explanation for the lack of toxicity of orally administered thiamin (Hayes and Hegsted 1973; LSRO 1978). The absence of adverse effects, aside from a rare allergic reaction after repeated daily doses of 100 mg injected intravenously, argues for an inherently low order of toxicity for thiamin.

Official Reviews

IOM (1998). The IOM found no data to identify a LOAEL for oral thiamin in either humans or animals. Thus, with no adverse effects from oral thiamin that would support selection of a LOAEL or specific NOAEL value, the IOM did not set a UL.

European Commission, Scientific Committee on Food (EC SCF 2001). The EC SCF found evidence of adverse effects only for injected thiamin. Since it found none for oral thiamin, it saw no need to set a UL value.

EVM (2003). The UK's EVM found that a small clinical trial (Meador et al. 1993) revealed no adverse effects of thiamin at daily oral intakes of 6,000 to 8,000 mg for 5 to 6 months. Based on a clinical trial with 556 young women given 100 mg thiamin for 60 to 90 days (Gokhale 1996), the EVM found no evidence of adverse effects at any level of intake; therefore, it set 100 mg per day as the guidance level for supplemental thiamin.

CRN Recommendations

CRN identifies a UL of 100 mg supplemental thiamin hydrochloride per day, based on clinical trial data (Gokhale 1996). The safe use of thiamin products at much higher levels, in addition to the clinical trial data of Meador and colleagues (1993), strongly suggests that much higher levels of thiamin are safe, but there is insufficient data to firmly support that conclusion.

Quantitative Summary for Thiamin (Vitamin B_1)

CRN UL, supplemental intake	100 mg/day
IOM UL, total intake	Not determined
EC SCF UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	100 mg/day

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Vitamin B_2 (Riboflavin)

Introduction

Riboflavin, like thiamin and some other B vitamins, is essential for normal development, growth, reproduction, lactation, physical performance, and well-being. It is involved in a wide array of essential biochemical oxidation-reduction reactions, especially those that yield energy and metabolize carbohydrates, fats, and proteins. Riboflavin is widely distributed in small amounts in many foods, and milk is one important dietary source. Similar to many members of the water-soluble B-complex family of vitamins, riboflavin is easily lost from grains or vegetables during milling, heating, canning, blanching, and storage. Riboflavin is especially sensitive to light. It is readily absorbed in small amounts from the intestine and readily excreted through the kidneys (McCormick 1999).

Safety Considerations

Riboflavin consumed orally has no reported toxicity (Miller and Hayes 1982; Institute of Medicine [IOM] 1998; Expert Group on Vitamins and Minerals [EVM] 2003). Reports of adverse effects all relate to animal studies or cell culture research involving either drugs with phototoxicity, intense exposure of lens tissue to ultraviolet light, or both in combination with high levels of riboflavin (Floersheim 1994; Spector et al. 1995). There are no reports of adverse reactions that can be attributed to riboflavin consumed orally from foods or dietary supplements.

Official Reviews

Institute of Medicine (IOM 1998). The IOM found no evidence of adverse effects associated with excess intake of riboflavin from food or supplements, that is, no toxicity data on which to base a LOAEL or a NOAEL. After extensive clinical and scientific investigation with elevated doses, the IOM found no basis for a UL value.

European Commission's Scientific Committee on Food (EC SCF 2000). The SCF found no evidence of toxicity of oral vitamin B_2 and did not set a UL.

Expert Group on Vitamins and Minerals (EVM 2003). Based on clinical trial data generated after the IOM review (Schoenen et al. 1998), the UK's EVM tentatively concluded that 400 mg per day produced only minor and infrequent side effects of uncertain significance. Because of the small number of subjects studied at that level of intake under controlled conditions, the EVM assigned the default toxicological

uncertainty factor of 10 and set a supplemental guidance level at 40 mg, with a total intake guidance level at 43 mg because intakes of riboflavin from conventional foods are 3.3 mg or less.

CRN Recommendations

Using the data of Schoenen and colleagues (1998), CRN identifies 400 mg per day of vitamin B_2 as a level that does not produce a significant pattern of adverse effects. The minor and inconsistent adverse effects reported with 400 mg supplemental intake suggest that the EVM uncertainty factor of 10 is unnecessarily restrictive. Hence, CRN identifies a NOAEL from the 400 mg LOAEL and considers an uncertainty factor of 2 to be sufficient. The widespread market presence of 200 mg riboflavin supplements without reported adverse effects is consistent with safety at this level.

Quantitative Summary for Vitamin B_2 (Riboflavin)

CRN UL, supplemental intake	200 mg/day
IOM, total intake	Not determined
EC SCF, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level	40 mg/day supplemental intake; 43 mg/day total intake

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Niacin: Nicotinic Acid, Nicotinamide, and Inositol Hexanicotinate

Introduction

Niacin is the vitamin B_3 and has fundamental roles as part of reduction/oxidation coenzymes involved in energy metabolism, amino acid metabolism, and detoxification reactions for drugs and other substances. Niacin comes from in several forms: (1) nicotinic acid (pyridine-3-carboxylic acid) (Burgeois et al. 2006), (2) nicotinamide (nicotinic acid amide), and (3) other derivatives (e.g., inositol hexanicotinate) "that exhibit the biological activity of nicotinamide" (Institute of Medicine [IOM] 1998). Other derivatives may be converted to nicotinic acid or may contain nicotinic acid, nicotinamide, or their releasable moieties; whether these compounds should be referred to as *niacin* depends on the biological effects that are attributed to them, the interpretation of the evidence for the rates of uptake and metabolism, and the release of the chemical components (apparent bioavailability) that produce biological effects similar to the primary forms of niacin.

Excess niacin is not stored in the body; therefore niacin must be ingested daily. It can come from nicotinic acid or nicotinamide. It can also come from the biological conversion of the amino acid tryptophan to nicotinic acid, but only if intake of protein is at a sufficiently high level. Thus, an individual's niacin level depends on the amount and quality of that person's dietary protein intake (Cervantes-Laurean et al. 1999).

Nicotinic acid at an intake of 1,000 mg or higher is an effective dyslipidemic agent with a broad spectrum of effects, including raising high-density lipoprotein (HDL) cholesterol, reducing low-density lipoprotein (LDL) cholesterol, reducing high lipoprotein(a), and reducing triglycerides (Witztum and Steinberg 1996; Carlson 2005). However, intakes of quantities of 1 g or more carry significant risk of adverse effects, ranging from nuisance effects to serious illness. For example, excessive intake of nicotinic acid can produce a vasodilative effect that results in an itching or burning sensation of the skin, especially on the face and neck. This "flushing reaction" usually persists for only a few doses and may be reduced by splitting the daily dose into three parts, each increased gradually until the desired total dose is achieved. More serious side effects impacting the liver or intestines have occurred when gram quantities are taken for dyslipidemia. Because of these risks, liver function tests and tests for uric acid, fasting blood glucose, and lipid levels should be conducted as part of the medical treatment with nicotinic acid. Adverse reactions may require decreased dosage or discontinuation in favor of other agents. Clinicians should be

aware that the flushing reaction may be substantially avoided through use of a "slow-release" preparation of nicotinic acid; however, such preparations carry greater risk of liver toxicity (Rader et al. 1992).

Nicotinamide, or niacinamide, performs all of the essential biochemical functions of niacin and prevents its deficiency. Large doses of nicotinamide do not cause vasodilatation or flushing and do not lower serum lipid concentrations.

Several forms of "niacin" are marketed in the United States as dietary supplements, including

- nicotinic acid (unmodified, immediate release)
- slow-release (extended release) forms that contain acid and an agent to slow the release (wax matrix, ion exchange gel, etc.)
- nicotinamide
- inositol hexanicotinate (IHN), described as "no flush niacin"

The bioavailability and safety considerations of each of these dietary supplements forms will be discussed and compared. One slow-release niacin product, Niaspan (nicotinic acid in an ion-exchange gel), is approved by the Food and Drug Administration (FDA) and marketed as a prescription drug for control of serum cholesterol and reduction of heart disease risk.

Bioavailability

Nicotinic Acid

Intestinal uptakes of free nicotinic acid are rapid and nearly total (IOM 1998); that is, single large doses of up to 3 to 4 g nicotinic acid are almost completely absorbed by adults (Bechgaard and Jespersen 1977). Once absorbed by the gut, up to 30 percent of the plasma nicotinic acid is bound to plasma protein. Nutritional functions related to niacin-dependent coenzymes occur at low intake levels (15 to 18 mg per day), whereas the flushing effect, which is a nuisance but is not dangerous, becomes noticeable when intakes exceed 50 mg per day (Spies et al. 1938). The beneficial effects reported on serum lipid profiles occur at much higher intake levels, such as 500 to 3,000 mg per day or more (Carlson 2005).

Extended-Release Nicotinic Acid

Extended-release nicotinic acid has been investigated for potential beneficial effects on serum lipids while minimizing or avoiding the flushing effect of crystalline nicotinic acid

(Norris 2006). For extended-release nicotinic acid, the potential impacts on serum lipid concentrations are directly related to the release of the nicotinic acid from the matrix in which it is presented. Several product technologies may be used to extend the release of nicotinic acid, such as an ion exchange and a wax matrix selected to melt slowly at body temperature. Thus, the uptake of nicotinic acid from extended-release nicotinic acid formulations is dependent on the specific delivery matrix and is significantly slower than that of free nicotinic acid, but rapid enough to achieve effective plasma nicotinic acid concentrations (Aronov et al. 1996; Menon et al. 2007).

Nicotinamide

Escalating oral doses of 3, 4, 5, 6, and 10 g of nicotinamide showed a linear relationship between maximum recorded plasma concentrations and the dose in grams. Maximum plasma levels were observed by 30 minutes in most patients ingesting up to 6 g of nicotinamide. Doses up to 6 g were well tolerated and resulted in average maximum recorded plasma levels (mean ± 1 SEM) of 156.4 ± 33.6 μg per ml. Doses of 10 g were generally not well tolerated, but a high plasma level was maintained on average for at least 4 hour (Dragovic 1995).

Inositol Hexanicotinate (IHN)

In 2009, the European Food Safety Authority (EFSA) Scientific Panel on Food Additives and Nutrient Sources Added to Food concluded that nicotinate from IHN is a bioavailable source of niacin (EFSA 2009). IHN, like extended-release nicotinic acid, has been investigated for potential beneficial effects on serum lipids while minimizing the flushing effect (Norris 2006). The available data suggest that intestinal absorption of IHN varies widely, with an average of 70 percent of the administered dose being absorbed into the bloodstream (Harthorn and Lindqvist 1964). However, the majority of IHN that is absorbed appears to remain intact after absorption. Possible direct actions of IHN after absorption have not been demonstrated but seem plausible.

Metabolism of IHN to release free nicotinic acid can result in the physiological functions of nicotinic acid, depending on the dose, rate, and amount of release. Beneficial lipid-lowering effects of free nicotinic acid and extended-release nicotinic acid are well established, but the beneficial effects of IHN on serum lipids would be dependent on uptake of IHN and subsequent release of the nicotinic acid moieties from the IHN molecule. The available reports indicate that IHN does not produce plasma nicotinic acid levels sufficient to lower lipids. Humans given oral doses of IHN obtain peak, but very low, levels of plasma free nicotinic acid at 6 to 12 hours (Welsh and Ede 1961; Sommer 1965), whereas oral doses of nicotinic acid result in peak plasma levels of nicotinic acid

at 0.5 to 1 hours (Carlson et al. 1968). The peak plasma levels of nicotinic acid after oral doses of IHN are dramatically lower compared with oral doses of nicotinic acid. For example, a single oral dose of 1,000 mg nicotinic acid resulted in a peak plasma level of 30 µg per mL nicotinic acid (Carlson et al. 1968), whereas 1,000 mg IHN (weight equivalent to approximately 910 mg nicotinic acid) resulted in a peak plasma level of 0.2 µg per mL nicotinic acid (Harthon and Brattsand 1979). Similarly, Kruse et al. (1979) gave 12 healthy young women 2,400 mg IHN orally over a 3-hour period and achieved a peak plasma nicotinic acid level of 0.1 µg per mL. Another experiment conducted in dogs compared the bioavailability of oral doses of 1 g of free nicotinic acid to the same amount of IHN and pentaerythritoltetranicotinate (INN). The peak plasma level for nicotinic acid was 130 times greater (approximately 65 µg per mL) than the peak plasma level for IHN (approximately 0.5 µg per mL) and 80 times greater than that for INN (0.8 µg per mL) (Harthon and Brattsand 1979).

Some reports indicate that IHN produces a slight increase in plasma nicotinic acid but does not have any significant effects on plasma lipid profiles (Harthon and Brattsand 1979). If IHN is absorbed intact and hydrolyzed in the body with the release of free nicotinic acid and inositol, the extent of hydrolysis appears to be very low. The significant differences in plasma levels of free nicotinic acid that are achieved after similar oral doses of IHN and free nicotinic acid may account for the different effects observed in clinical studies. In fact, the observed effects for IHN may not be related to its total nicotinic acid content, but rather a direct effect of IHN itself. Overall, the evidence indicates that IHN produces only slight increases in plasma nicotinic acid, but these changes are not large enough to significantly alter plasma lipid profiles (Meyers et al. 2003).

Safety Considerations

Nicotinic Acid and Extended-Release Nicotinic Acid

Nicotinic acid can produce a variety of adverse effects, depending on the intake and health of the consumer. The skin flushing reaction produced by nicotinic acid has been recognized for more than 70 years (Bean 1978). When taken on an empty stomach, crystalline nicotinic acid in doses as small as 10 mg may produce a mild and transient, but noticeable, flushing reaction. While not desirable, such reactions produce no known adverse consequences, and they are almost never perceptible when small amounts of nicotinic acid are taken in tablet or capsule form or consumed as part of food.

Serious side effects on the liver or intestines from nicotinic acid have occasionally occurred when gram quantities were taken to lower serum lipids (Rader et al. 1992).

Gastrointestinal side effects may include indigestion, nausea, vomiting, and diarrhea and, in some people, may necessitate discontinuation of nicotinic acid supplements. Liver toxicity clinically presents as increases in serum transaminase enzymes of liver origin released by damage to liver cells. Small increases in serum concentrations of transaminases do not indicate significant liver damage and return to normal after cessation of nicotinic acid intake. More severe reactions may produce jaundice, fatigue, and, in at least one case, fulminant liver failure (Clementz and Holmes 1987).

There is a strong correspondence between the minimum adverse effect level identified through clinical trials and that suggested by published anecdotal case reports. Many severe reactions to nicotinic acid, especially liver toxicity, have involved ill-advised, uninformed, or inadvertent switching from unmodified nicotinic acid preparations to extended-release formulations (Rader et al. 1992; MacKay et al. 2012). Most reported adverse reactions to nicotinic acid have occurred with intakes of 2,000 to 6,000 mg of elemental nicotinic acid per day in both unmodified and extended release forms. There are two anecdotal cases reported in which intake levels were below 1,000 mg: one for extended-release nicotinic acid at 500 mg per day and one for unmodified nicotinic acid at 750 mg per day (Rader et al. 1992). The clinical trial of McKenney et al. (1994) used two groups of adult subjects, studying one for immediate-release nicotinic acid and one for slow-release nicotinic acid. These two groups were observed for 6 weeks at dosage levels of 500, 1,000, 1,500, 2,000, and 3,000 mg per day. The data showed no adverse reactions at 500 mg per day for either form of nicotinic acid, but did show statistically significant effects beginning at 1,000 mg per day for both forms (e.g., gastrointestinal effects for unmodified nicotinic acid, and mild liver toxicity for slow-release nicotinic acid).

More recently, Grundy et al. (2002) studied extended-release nicotinic acid (the FDA-approved product Niaspan) in an attempt to control dyslipidemia in patients with type II diabetes. In this well-designed but modestly sized clinical trial, groups with placebo (n = 49), 1,000 mg extended-release nicotinic acid (n = 45), or 1,500 mg extended-release nicotinic acid (n = 52) were assessed for clinical benefits and monitored for adverse effects. Rates of adverse events other than flushing were similar for the niacin and placebo groups. Four patients discontinued participation owing to flushing, but one of these was in the placebo group. No hepatotoxic effects or myopathy were observed. This trial involved persons with type II diabetes, so the application to the general population is not certain.

Gram quantities of nicotinic acid should not be self-administered as a dietary supplement but may be safely used under the care and monitoring of a physician. Such application, it

should be noted, is a pharmaceutical use, not a dietary supplement use. It is especially important for individuals who want to achieve higher intakes of extended-release nicotinic acid to achieve these levels in a gradual step-wise manner.

Nicotinamide

Nicotinamide does not cause a flushing reaction. The 1998 study by the IOM's Food and Nutrition Board (FNB) study determined a LOAEL for vasodilatation and the flushing effect from nicotinic acid; but they then, unfortunately, applied that LOAEL to both nicotinic acid and nicotinamide. Later studies by EFSA and the EVM corrected this error.

IHN

IHN does not cause a flushing reaction. Clinical trials using IHN of up to 4,000 mg daily for 3 months do not demonstrate adverse effects (Sunderland et al. 1988). These clinical trials have not been designed to assess safety of IHN; however, no meaningful adverse effects have been noted in several well-designed clinical trials using IHN in amounts that range from 600 to 4,000 mg daily.

Official Reviews

IOM (1998). In 1998 the Food and Nutrition Board of the IOM, working in cooperation with scientists from Canada, published a comprehensive set of reference values for the B vitamins and choline for healthy U.S and Canadian populations. The panel members also reviewed data and applied risk assessment models to each B vitamin and choline to develop ULs. As noted above, the 1998 IOM report erroneously attributed the flushing effect from nicotinic acid to nicotinamide as well. In addition, it implicitly judged the flushing reaction to qualify as a "hazard" and therefore an appropriate basis for a UL, principally because of the undesirability of the effect rather than any evidence of actual harm. This nuisance caused by nicotinic acid was clearly illustrated by the accidental addition of the substance to bagel dough, resulting in uncomfortable and unexpected effects that were experienced by several persons who consumed the bagels (Centers for Disease Control 1983). Clearly, this flushing effect is less acceptable in ordinary foods than in dietary supplements because the latter can carry label statements to inform the consumer of this likely effect.

The LOAEL identified by the IOM was 50 mg, based on the clinical studies by Sebrell and Butler (1938) and Spies et al. (1938). Because of the mild and transient nature of the flushing effect, the IOM justified a UF of 1.5 to apply to the LOAEL, leading to determination of a UL of 35 mg. The 35 mg dose, however, may trigger the flushing

reaction in a few persons. It is noteworthy that the clinical studies from which the IOM derived the UL value involved bolus doses of nicotinic acid administered to subjects with empty stomachs and no previous regular exposure to dosed nicotinic acid, thus increasing the likelihood of this undesirable effect. The 1998 IOM report did not evaluate the UL for IHN.

European Commission, Scientific Committee on Food (EC SCF 2002). In 2002 the EC SCF published its report on nicotinic acid and nicotinamide, which is one in the series of opinions on the upper levels of vitamins and minerals. The SCF report recognized that the more severe forms of toxicity of nicotinic acid occur only at doses greater than 500 mg but identified a LOAEL of 30 mg, based on the skin flushing reaction in the same studies (i.e., Sebrell and Butler 1938; Spies et al. 1938) relied upon by the IOM. Regardless of the lower LOAEL identified by the EC SCF on the same studies used by the IOM, they selected a larger UF of 3 and therefore derived a UL of 10 mg for nicotinic acid. The EC SCF attempted to justify its identification of the vasodilatory (flushing) effects as the critical adverse effect endpoint (that is, as the *hazard* of concern in the UL risk assessment model) based not only on the nuisance of discomfort but also on the purely hypothetical possibility of exaggeration of positional hypotension and a possibly related increased risk of falls, which are a common cause of morbidity and mortality in the elderly. No evidence has emerged to support this supposition during the decade since this review was published.

For nicotinamide, the EC SCF identified a UL of 900 mg. This value has a substantial margin of safety built in to identify this UL as a value well below the clinical trial values that showed no adverse effects. The 2002 SCF report did not evaluate the UL for IHN.

Expert Group on Vitamins and Minerals (EVM 2003). The EVM's 2003 report concluded that "there are insufficient data from human or animal studies to establish a safe upper level for nicotinic acid." Nonetheless, the EVM set a guidance level for nicotinic acid based on UL methodology applied to animal data. In apparent disagreement with the IOM on a LOAEL of 50 mg (based on Sebrell and Butler 1938 and Spies et al. 1938) and with the EC SCF on a UF of 3, the EVM derived a unique guidance level of 17 mg for nicotinic acid.

Like the EC SCF, the EVM established a safe level for nicotinamide distinct from that for nicotinic acid. It identified no adverse effects for nicotinamide at intakes of 25 mg per kg (Pozzilli et al. 1995) and 42 mg per kg (Lampeter et al., 1998), but judged the database small enough to justify a UF of 3. The derived guidance level for a 60-kg person is 500 mg of supplemental nicotinamide per day. Assuming a food intake of not more than 57

mg from foods, the EVM identified 560 mg per day as the guidance level for total intake of nicotinamide from all sources. The EVM did not establish a guidance level for IHN.

CRN Recommendations

Nicotinic Acid

With its transient and nonpathological effects, the flushing reaction in response to supplemental nicotinic acid deserves to be characterized as a *nuisance*, but not as a *hazard.* When high intakes result from supplementation, appropriate product labeling can alert the consumer of the flushing effect. Thus, flushing does not qualify as a hazard for supplemental intakes of nicotinic acid. The CRN UL for excessive supplemental nicotinic acid is based on the hepatotoxic effects at much higher doses, effects that can be clearly hazardous.

There are only two anecdotal cases of reported hepatotoxic effects at intakes less than 1,000 mg per day, and many uncertainties exist in these cases regarding the amount consumed as well as the presence or absence of pre-existing or confounding conditions such as alcoholism or other compromises of liver function. The clinical trial data of McKenney et al. (1994) are appropriate to identify a NOAEL of 500 mg per day and a LOAEL of 1,000 mg per day for liver or gastrointestinal effects. It should be noted, however, that the adverse reactions to 1,000 mg of unmodified nicotinic acid were mainly gastrointestinal effects, which generally have less potential for serious outcomes, rather than the liver toxicity that results in some persons consuming 1,000 mg per day of slow-release nicotinic acid. With proper labeling, consumers can be aware of gastrointestinal effects and correct as needed. These differences warrant advising a lower limit for slow-release nicotinic acid than for the unmodified form, and the twofold decreases in the NOAEL and LOAEL for slow-release nicotinic acid seem ample, based on case reports (Rader et al. 1992) and clinical trial results (McKenney et al. 1994). Thus, the NOAEL is 250 mg and the LOAEL is 500 mg for slow-release nicotinic acid.

The reports by the IOM, the EC SCF, and the EVM did not set NOAEL or LOAEL values based on the hepatotoxic effects of nicotinic acid. Those reviews identified intakes of about 3 g as the levels at which such effects occur with substantial frequency. None of these reports addressed slow-release nicotinic acid preparations in any detail.

The results by Grundy et al. (2002) challenge the validity of the assumption that extended-release nicotinic acid is necessarily more toxic than crystalline nicotinic acid if used in an appropriate manner, with a gradual escalation of the dose and careful clinical monitoring. It may be very important in the clinical trial by Grundy et al. that the highest

doses of extended-release nicotinic acid were achieved by weekly step-wise increases (e.g., 375 mg extended-release nicotinic acid at bedtime in week 1; 2,500 mg in week 2; 750 mg in week 3; and 1,000 mg in week 4). These results indicate that 1,000 mg extended-release nicotinic acid can be safely consumed with a step-wise escalation of intake and medical supervision.

Nicotinamide

There is much less information on nicotinamide than there is for nicotinic acid, but there also appears to be much less use at high levels of intake. Clinical trials on high-dose nicotinamide have been small. One study observed no adverse effects in 16 subjects who received 3,000 mg of nicotinamide per day (Vague et al. 1987), but the method of monitoring for such effects was not specified. Other studies that describe monitoring methods in more detail have found no adverse effects for nicotinamide intakes in the range of 1,000 to 2,900 mg per day (Mendola et al. 1989; Chase et al. 1990; Pozzili et al. 1995; Lampeter et al. 1998). Nicotinamide intakes of more than 3,000 mg per day have resulted in adverse gastrointestinal and liver effects (Rader et al. 1992).

The clinical trial results support a very confident NOAEL of 25 mg per kg per day. Because some of these trials were performed with subjects aged younger than 18 years who had lower than fully adult body weights, 60 kg was used to calculate a NOAEL of 1,500 mg per day. The absence of adverse effects in clinical trials that included nicotinamide dosages of up to 3,000 mg per day reduces the uncertainty in this value.

IHN

Several clinical studies have demonstrated that IHN may have a beneficial effect on endothelium-dependent vasodilatation. The clinical research literature includes several positive studies on the use of IHN for improving blood flow in conditions where blood flow is compromised (Ring and Bacon 1977; Head 1986; O'Hara et al. 1988). IHN is prescribed in Europe as a patented drug known as Hexopal, which is therapeutically indicated for the symptomatic relief of severe intermittent claudication and Raynaud's phenomenon. The usual adult dose of IHN for these conditions is 3 g per day and is increased to 4 g per day if necessary (Genus Pharmaceuticals 2008). Clinical trials using IHN range from 600 to 4,000 mg daily. No adverse effects have been identified in clinical trials even when 4,000 mg per day IHN was administered orally to humans for 3 months (Sunderland et al. 1988). Clinical trials on IHN have not been specifically designed to assess safety of IHN; however, no meaningful adverse effects have been noted in several well-designed clinical trials. These clinical trials support a NOAEL of

4,000 mg. The absence of observed adverse effects does not support the establishment of a LOAEL.

CRN identifies the following LOAEL and NOAEL values for niacin supplements:

Immediate-Release Nicotinic Acid Formulations	
LOAEL	1,000 mg/day
NOAEL	500 mg/day
Flushing label warning threshold	>35 mg/day
Slow-Release Nicotinic Acid Formulations	
LOAEL	500 mg/day
NOAEL	250 mg/day (without step-wise escalation); 500 mg/day (with step-wise escalation of intake over a few weeks)
Flush label warning threshold	Not needed
Nicotinamide	
LOAEL	3,000 mg/day
NOAEL	1,500 mg/day
Flush label warning threshold	Not needed
Inositol Hexanicotinate (IHN) Formulations	
LOAEL	Not established
NOAEL	4,000 mg/day
Flush label warning threshold	Not needed

Considering the infrequent occurrence at the LOAEL levels of intake and the reversible nature of mild, short-term hepatotoxicity, the NOAEL values are identified as the CRN UL values for supplements, provided that immediate-release formulations carry appropriate labeling.

Quantitative Summary for Nicotinic Acid

CRN UL, supplemental intake —immediate release	500 mg/day (based on liver effects)
CRN UL, supplemental intake —based on flushing effect	35 mg/day (no label statement needed)
CRN UL, supplemental intake —slow-release niacin	250 mg/day (step-wise increases)
IOM UL, total intake	35 mg/day (based on flushing effects)
EC SCF UL, total intake	10 mg/day (based on flushing effects)
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	17 mg/day (based on flushing effects)

Quantitative Summary for Nicotinamide

CRN UL, supplemental intake	1,500 mg/day
IOM UL, total intake	35 mg/day
EC SCF UL, total intake	900 mg/day
EC supplement maximum	Not determined
EVM, guidance level	500 mg/day supplemental intake; 560 mg/day total intake

Quantitative Summary for Inositol Hexanicotinate

CRN UL, supplemental intake	4,000 mg/day
IOM UL, total intake	Not determined
EC SCF UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level	Not determined

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Vitamin B_6 (Pyridoxine)

Introduction

Vitamin B_6 is a water-soluble vitamin that is important in carbohydrate, lipid, and amino acid metabolism. It is found in the body in three primary forms: pyridoxine (the common name given mainly to the alcohol form, or pyridoxol); pyridoxal (the aldehyde); and pyridoxamine (the amine). The activated forms of pyridoxal and pyridoxamine are active coenzyme forms, and the interconversion between them is involved in many of the biological functions of vitamin B_6. This vitamin is extensively involved in the metabolism of nitrogen-containing compounds, including serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and the heme component of hemoglobin. Pyridoxine, as pyridoxal phosphate, also has an important role in the conversion of tryptophan to nicotinic acid.

Vitamin B_6 interacts with several drugs, which may either decrease the activity of the drug or increase the need for the vitamin or both. Individuals taking medications on a regular basis should discuss their vitamin B_6 status with their health care providers. Furthermore, several medical conditions, including autoimmune disorders, impaired renal function, and alcohol dependence, can increase the requirement for pyridoxine (Institute of Medicine [IOM] 1998; Bates 1999; Chiang 2005).

Safety Considerations

Both deficiency and excess of pyridoxine may produce neurological disturbances (Hathcock and Rader 1990). The first report of pyridoxine neurotoxicity in humans described a sensory neuropathy of the extremities in women with daily intakes of 2,000 to 6,000 mg, mostly taken in attempt to control premenstrual symptoms (Schaumburg et al. 1983). The neuropathy slowly and often incompletely regresses after cessation of the elevated dose (Albin et al. 1987; Albin and Albers 1990; Santoro et al. 1991). Most cases of sensory neuropathy have resulted from intakes of greater than 600 mg per day, but evidence suggests that for some individuals, neuropathy may occur after doses as low as 300 to 500 mg (Parry and Bredesen 1985; Bendich and Cohen 1990; Hathcock and Rader 1990). At high intake levels, the total dose over time may give a better prediction of the potential for neurotoxic response than either the daily dose or the duration of the high intake (Bendich and Cohen 1990). This relationship, however, does not extend to low, nontoxic doses.

Treatment with either 150 or 300 mg of pyridoxine for up to 4 months did not produce signs of sensory neuropathy or any other adverse effects in 24 carpal tunnel syndrome patients (Del Tredici et al. 1985). Bernstein and coworkers (Bernstein and Lobitz 1988; Bernstein and Dinesen 1993), using physical neurological methods, found no evidence of neurological effects of pyridoxine at intakes of up to 200 mg per day over a period of 4 months. Most subjects showed no adverse effects at 150 to 200 mg per day supplemental intake (Del Tredici et al. 1985; Parry and Bredesen 1985; Bernstein and Lobitz 1988; Bernstein and Dinesen 1993). At intakes of 200 mg (but not at 150 mg), a few subjects experienced signs of adverse neurological effects such as sensory tingling and numbness (Parry and Bredesen 1985; Brush 1988). Consumption of 200 mg pyridoxine per day may decrease the time it takes for adverse effects to develop after higher levels are initiated (Parry and Bredesen 1985).

A double-blind, placebo-controlled study in which 100 or 500 mg of vitamin B_6 was consumed daily for 14 days showed marginal evidence of improvement in a digital coding test but also some evidence of an adverse effect on word recognition (Molimard et al. 1980); no further evidence to support either of these possible effects has been published. The apparent adverse effect was significant at a 500 mg intake level, but not at 100 mg.

There is strong controversy over the validity of the study reporting of adverse effects at daily pyridoxine intakes of around 100 mg or less (Dalton and Dalton 1987). The design of the study, which involved telephone interviews using leading questions, has raised questions about the validity of its observed effects. Although the Dalton and Dalton report has been cited as evidence that pyridoxine intakes below 100 mg per day can cause sensory neuropathy (European Commission's Scientific Committee on Food [EC SCF] 2000; Expert Group on Vitamins and Minerals [EVM] 2003), the data showed an average intake of 117 mg per day among women with adverse symptoms and a nearly identical average intake (116 mg per day) in the control group. The group with reported symptoms had taken pyridoxine for a longer period of time—an average of 2.9 years, compared with 1.6 years for those without symptoms. Some women reporting adverse effects had intakes of 50 mg or less. Inaccuracies in the telephone survey method and a lack of objective neurological assessment are likely to have introduced bias. The symptoms observed had no dose-response relationship to pyridoxine intake, but they did show a time-response relationship. If the time-dose relationship were extended far enough it would lead to the conclusion that even deficient intakes would be "toxic" if taken long enough. The IOM concluded that the data were not of sufficient quality to warrant use in a risk assessment for pyridoxine (IOM 1998).

Some reports have suggested that high intakes of pyridoxine may carry risk of oxalate kidney stones, but these reports are problematic. The reported cases may have been associated with the drug pyridoxilate (a combination of pyridoxine and glyoxalate) (Daudon et al. 1987), and a recent prospective epidemiological study found the relative risk of oxalate renal stones to be decreased for men consuming more than 40 mg of pyridoxine in comparison with those consuming less than 3 mg (Curhan et al. 1996).

There seems to be no recent publications on the neurotoxic effects of pyridoxine, but there are many reports of this vitamin influencing the effects of toxic compounds, for example, cadmium (Wen et al. 2010).

Official Reviews

IOM (1998). The IOM identified a NOAEL of 200 mg from clinical data (Del Tredici et al. 1985; Bernstein and Lobitz 1988) but considered the Dalton and Dalton (1987) data too unreliable to serve as the basis of a UL. It thus applied a UF of 2 to the 200 mg human NOAEL, deriving a UL of 100 mg.

EC SCF (2000). While the EC SCF recognized the weaknesses of the Dalton and Dalton data, it considered the other available clinical data to be of marginal scientific quality as well. Consequently, it used the Dalton and Dalton data as the basis of its pyridoxine UL, dividing an intermediate LOAEL of 100 mg per day by a composite UF of 4. This 100 mg LOAEL was formulated from the intakes of the Dalton and Dalton group with adverse effects, which consumed a mean intake of 117 mg and a median intake of <100 mg. (The Dalton and Dalton report asserted that some individuals with minor adverse effects had taken only 50 mg per day, but this was not factored into the EC SCF LOAEL.) The composite UF of 4 resulted from assigning a factor of 2 to account for long-term intakes and a further factor of 2 to allow for deficiencies in the database. The EC SCF UL of 25 mg per day that resulted from the selected LOAEL and UFs was justified by the absence of any reports, even anecdotal ones, of adverse effects at intakes of 25 mg per day.

EVM (2003). The UK's EVM, concluding that the available human data were inadequate, based their assessment on a study that found a LOAEL of 50 mg per kg body weight per day in dogs (Phillips et al. 1978). They extrapolated the dog data to a 60-kg human adult representative weight by applying a composite UF of 300 to derive an SUL of 10 mg per day. The composite factor represented a factor of 3 for LOAEL-to-NOAEL extrapolation, a factor of 10 for interspecies extrapolation, and another factor of 10 for variation in human sensitivity.

In summary, these three government reports based their risk assessment on widely differing datasets and methods, especially in determining uncertainty. The result is that the daily amounts of pyridoxine considered safe differ significantly: 100 mg for the IOM, 25 mg for EC SCF, and 10 mg for the EVM. These disparate outcomes suggest that better data selections and uncertainty assessment are needed and that current corresponding UL values are somewhat arbitrary.

CRN Recommendations

The complete absence of adverse effects in credible, well-designed studies at 100 and 150 mg and only marginal evidence of adverse effects at 200 mg (Parry and Bredenson 1985; Brush 1988) indicate that 100 mg can be confidently identified, with a low level of uncertainty, as a safe level of consumption. Consequently, CRN identifies the supplemental intake NOAEL for pyridoxine to be 100 mg. Intakes from conventional foods alone are generally below 3 mg (IOM 1998; EVM 2003), and thus this source does not meaningfully contribute to safety concerns. CRN's UL is 100 mg, but somewhat higher amounts may be safe for most people and/or for shorter periods of time.

Quantitative Summary for Vitamin B_6 (Pyridoxine)

CRN UL, supplemental intake	100 mg/day
IOM UL, total intake	100 mg/day
EC SCF UL, total intake	25 mg/day
EC supplement maximum	Not determined
EVM SUL, supplemental intake	10 mg/day

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Folic Acid

Introduction

The term "folic acid" is used to denote pteroylmonoglutamic acid or vitamin B_9. "Folate" indicates the naturally occurring compounds in foods that are aromatic pteridine rings linked to para-aminobenzoic acid with 2 to 8 glutamic acid groups attached to the primary structure (Herbert 1999). Folic acid is used as a dietary component (i.e., in food fortification and dietary supplements) but must be metabolized to the reduced dihydrofolate and tetrahydrofolate forms for biological activity. The active (dihydrofolate and tetrahydrofolate) forms of folic acid are involved in a wide variety of biochemical reactions, particularly one-carbon metabolic reactions. A deficiency of folic acid impairs DNA synthesis and cell division; the common clinical manifestation of severe folic acid deficiency is megaloblastic (larger than normal but fewer red blood cells) anemia, which is hematologically similar to the anemia resulting from vitamin B_{12} deficiency.

There is clear evidence that sufficient maternal dietary folic acid before conception and very early in pregnancy (within the first 28 days postfertilization) can decrease the risk of having babies with neural tube birth defects (NTDs), which include spina bifida, anencephaly, and encephalocele (Food and Drug Administration [FDA] 1993a, 1993b). All the clinical trial evidence showing a reduced risk of NTDs relates to supplemental folic acid, but the health claim authorized for the United States by the FDA is related to "total folate," or naturally occurring food folates plus folic acid from fortified foods or dietary supplements. The daily intake of folic acid shown to be effective for this purpose is 400 μg (0.4 mg) or higher, an amount above the RDA in most countries. Although folic acid can reduce the risk for NTDs, these defects are not solely attributable to folic acid deficiency. Folic acid supplementation generally reduces the risk by 50 to 75 percent.

Preliminary evidence suggests that sufficient dietary folic acid can decrease the plasma concentration of homocysteine, a substance that is gaining scientific recognition as a causative risk factor for heart disease (Boushey et al. 1995; den Heijer et al. 1996; Malinow 1996; Institute of Medicine [IOM] 1998). The data are not yet sufficient to make a reliable estimate of the amount of folic acid needed to generate the health benefits, but the levels identified as possibly effective are in the same range as those shown to be effective against NTDs.

Bioavailability

Food folates must be deconjugated—that is, most glutamic acid groups must be removed from them—by the intestinal enzyme folate conjugase before absorption can occur; after absorption, reduction to the dihydrofolate or tetrahydrofolate forms is necessary for biological activity. Following absorption, dietary folic acid is activated in the same manner as diet-derived folates. The folic acid activity of dietary folates and folic acid depends on the efficiency of absorption, the efficiency of conversion of folates to folic acid, and the relative molecular weights of food folates and folic acid. Currently, folate requirements are expressed as dietary folate equivalents (DFE) (IOM 1998), with 1 µg DFE equal to 1 µg of food folates, 0.5 µg of folic acid taken on an empty stomach, or 0.6 µg of folic acid taken with meals. These differences reflect the relative bioavailability of folic acid versus folate; folic acid added to foods during fortification or supplementation is 70 to 85 percent bioavailable compared with 50 percent of folate occurring naturally in foods (Hoyo et al. 2011).

Safety Considerations

No adverse effects have been associated with consumption of food folates or folic acid in fortified foods or dietary supplements (IOM 1998). Folates and folic acid are water soluble and thus excretion is relatively straightforward. Three primary concerns have been identified as possible adverse effects from excessive levels of supplemental folic acid intake: (1) the masking of pernicious anemia, which allows the neurological disease of vitamin B_{12} deficiency to progress unchecked; (2) the disruption of zinc function; and (3) the antagonism of medications, especially antifolate agents such as methotrexate. Each of these consequences presents serious concerns and warrants careful consideration of the evidence. The evidence is weak to nonexistent that folic acid has adverse effects by any mechanism other than these three (Campbell 1996; European Commission, Scientific Committee on Food [EC SCF] 2000).

Neurological Effects from Masking of Vitamin B_{12} Deficiency

The administration of high levels of folic acid to patients with pernicious anemia can mask anemic manifestations while allowing neurological disease (posterolateral spinal cord degeneration) to progress (Butterworth and Tamura 1989; IOM 1998; National Institutes of Health [NIH] 2012). Fortunately, this devastating complication is not known to occur with the amounts of folic acid intake obtained through ordinary diets or through the levels of intake contained in the vast majority of dietary supplements. The more convincing reports of the masking effect involve administration of 5 mg or more folic acid per day. A few early reports showed some response in certain hematological indices

for pernicious anemia patients taking folic acid doses as low as 0.1 to 0.8 mg. These effects are sometimes interpreted as indicating possible risk from increased folic acid intakes (Savage and Lindenbaum 1995). The risk, however, is speculative because more than 25 percent of vitamin B_{12}-deficient patients who are not taking folic acid did not have anemia (normal hematocrit and normal mean cell volume) but only neurological signs (Healton et al. 1991). Thus, a report of an individual with neurological signs of vitamin B_{12} deficiency who has also taken folic acid supplements (Brantigan 1997) does not conclusively show evidence of a masking effect.

There is no clear evidence that folic acid changes the time course or neurological outcome of vitamin B_{12} deficiency. Although there are a few reports of an incomplete masking effect resulting from amounts of folic acid smaller than 1 mg, the effect is unusual at that intake and is predictable only at 5 mg or more (FDA 1993a, 1993b). In addition, many pernicious anemia patients who respond to folic acid may also be deficient in folic acid (Dudley and Coltman 1970). Hemoglobin and hematocrit respond to folic acid administration in some patients, particularly those receiving folic acid in high oral doses or through parenteral administration. Regardless of this effect, folic acid does not completely normalize hematological morphology in vitamin B_{12} deficiency (Herbert 1963).

Folic Acid–Zinc Interactions

Certain folic acid–zinc interactions are well documented. The folate conjugase enzyme must act on food pteroylpolyglutamates for absorption, which is reduced in zinc deficiency (Butterworth and Tamura 1989). The crucial issue, however, is whether higher intakes of folic acid have adverse consequences through a disruption of zinc bioavailability or function and, if so, what the levels of folic acid associated with such effects are. Some reports suggest that as little as 350 µg of supplemental folic acid can adversely affect zinc nutriture (Milne et al. 1984; Mukherjee et al. 1984; Simmer et al. 1987), but more recent reports indicate no adverse effects of folic acid on zinc uptake or function (Tamura et al. 1992; Kauwell et al. 1995).

The suggestion that folic acid intakes of less than 400 µg (0.4 mg) per day can negatively affect pregnancy through the antagonism of zinc functions (Mukherjee et al. 1984) was not supported by a large, multicenter study involving a tenfold higher folic acid intake throughout pregnancy (Wald et al. 1991).

It is difficult to resolve differences in the scientific literature regarding a possible adverse effect of folic acid on zinc nutriture. The incompatible results can likely be attributed to

the widely different experimental approaches used. In general, methods based on uptake rate and plasma concentration tend to show effects at lower folic acid intakes, whereas zinc balance methods tend to show effects only at higher intakes. Large, well-conducted clinical trials have found no adverse effects of folic acid on pregnancy through zinc antagonism or any other mechanism, but they have demonstrated a clear benefit in reducing the risk of NTDs (Wald et al. 1991; Czeizel and Dudas 1992).

Folic Acid–Drug Interactions

At very high levels of intake, folic acid has been reported to interfere with the effectiveness of anticonvulsant drugs such as diphenylhydantoin, which is used in controlling epilepsy (FDA 1993a, 1993b). Oral folic acid doses of 5 to 30 mg have produced some evidence of increased frequency of seizures in epileptics, but there is no evidence of such effects at lower intakes of folic acid. It might be expected that increased folic acid intakes could interfere with actions of folate antagonistic drugs such as methotrexate. Conversely, administration of 1 mg folic acid daily for 6 months in patients with rheumatoid arthritis who were treated with low-dose methotrexate actually decreased methotrexate toxicity without affecting the drug's therapeutic efficacy (Morgan et al. 1990).

Official Reviews

IOM (1998). The IOM established a UL of 1,000 μg for free folic acid, based on identification of a LOAEL of 5,000 μg and selection of a UF of 5. The LOAEL was based on neurological manifestations in patients receiving 5 mg or more folic acid without supplemental vitamin B_{12}. The IOM declined to identify a NOAEL, although many of the studies it cited failed to find adverse effects at doses of 1 to 1.25 mg folic acid. There is no record of adverse effects caused by food polyglutamyl folates, perhaps because of the lower bioavailability and/or the limited range of intakes observed. No studies have been done with elevated doses of purified polyglutamyl folates. Thus, the UL applies to purified folic acid only, either in fortified foods or in dietary supplements.

EC SCF (2000). Like the IOM, the EC SCF established a UL of 1,000 μg for folic acid, basing its finding apparently on both a LOAEL of 5,000 μg and a UF of 5, and also on a NOAEL of 1,000 μg and a UF of 1 (EC SCF 2000). In addition to identifying adverse effects at dosages above 5 mg, the EC SCF concluded that "dosages of up to 1 mg of folic acid are unlikely to cause masking of the hematological signs in PA patients." The resulting UL is 1,000 μg of free folic acid, but this value does not apply to the polyglutamyl folates found naturally in foods.

Expert Group on Vitamins and Minerals (EVM 2003). Similar to the IOM and the EC SCF, the UK's EVM established a guidance level of 1,000 µg of free folic acid (EVM 2003). This value was based on both a NOAEL of 1 mg and a LOAEL of 5 mg, with UF values applied that produced the UL of 1,000 µg. The EVM considered the entire dataset to be uncertain enough to preclude setting SUL values, but its guidance level was derived using the SUL method and was applied in that manner.

CRN Recommendations

A folic acid supplement of 4 mg per day (4,000 µg) was used without adverse effect in a seven-nation trial that involved a total of 1,817 women at 33 study centers (Wald et al. 1991). A committee advising the FDA on folic acid and NTDs concluded that adverse effects were unlikely with intakes of 1,000 µg (1 mg) or less (FDA 1996). The evidence that intakes of 1,000 µg (1 mg) of total folic acid plus food folates are without identifiable risk of any known adverse effects is sufficient to identify this level as the NOAEL. This conclusion is consistent with the advice of the FDA's Food Advisory Committee and the U.S. Public Health Service, but may be more related to cautious policy than to scientific evidence. Reports of adverse effects from lower intakes of folic acid have been contradicted by subsequent studies, and therefore these reports are not useful in the identification of a NOAEL or a LOAEL. Some data suggest that the LOAEL might be 5,000 mg.

The two studies found no significant increase in risk of masking neurological effects with folic acid doses of 1.25 mg per day (Ross et al. 1948; Chodos and Ross 1951), whereas there is some evidence that masking may be a problem with intakes of 1.5 and 2.55 mg (Victor and Lear 1956). On the basis of the absence of adverse effects at 1,000 µg and no significant effects up to 1.25 mg, CRN sets its UL for supplemental folic acid at 1,000 µg. The identification of a LOAEL at 5,000 µg by the IOM, the EC SCF, and the EVM—together with the absence of any data that would suggest a LOAEL lower than 1.5 or 2.55 mg—provides a margin of safety to allow for intakes of folic acid–fortified foods. Therefore the 1,000 µg NOAEL may be applied to supplemental folic acid, making the CRN UL for supplements of folic acid 1,000 µg.

Quantitative Summary for Folic Acid

CRN UL, supplemental intake	1,000 µg/day
IOM UL, total intake	1,000 µg/day
EC SCF UL, total intake	1,000 µg/day
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	1,000 µg/day

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Vitamin B_{12}

Introduction

Vitamin B_{12} helps maintain the body's nervous system and blood cells and supports the production of DNA. Vitamin B_{12} also helps prevents a type of anemia and has been termed the "anti-pernicious anemia dietary factor." Vitamin B_{12} is also the only known physiologically important compound that contains cobalt, and therefore the various forms of vitamin B_{12} are known collectively as cobalamins.

Vitamin B_{12} is a cofactor in two enzymes that are fundamental in facilitating growth in humans. In the methylcobalamin form, vitamin B_{12} is the direct cofactor for methionine synthetase, the enzyme that recycles homocysteine back to methionine. Here, vitamin B_{12} and folic acid have closely related roles in one-carbon metabolism. In the adenosylcobalamin form, vitamin B_{12} is the cofactor in methylmalonyl-coenzyme A mutase. Both reactions are involved in promoting the rapid growth and proliferation of bone marrow cells and ultimately red blood cells (Expert Group on Vitamins and Minerals [EVM] 2003).

Vitamin B_{12} is essential for the function and maintenance of the central nervous system, and severe deficiency in persons with pernicious anemia produces the neurological disease of posterolateral spinal cord degeneration (Herbert and Das 1994). The direct cause of pernicious anemia, in fact, is vitamin B_{12} deficiency, but the underlying defect is the absence of an intrinsic factor produced by specific stomach cells and needed for intestinal absorption of vitamin B_{12}. Without this intrinsic factor, absorption is greatly reduced or fails, and a severe and persistent deficiency develops that is not preventable by the usual dietary levels of vitamin B_{12}. In addition to the efficient, intrinsic factor-mediated absorption of small quantities of the vitamin from normal dietary intakes of up to about 6 µg, there is also a very low efficiency of absorption of much higher oral intakes (300 to 1,000 µg). Therefore, high daily oral intakes can be sufficient to treat pernicious anemia by utilizing high intake levels coupled with low efficiency absorption resulting in adequate serum levels. However, the usual treatment is a monthly vitamin B_{12} intramuscular injection, which bypasses intestinal absorption and the requirement for intrinsic factor for absorption (Hathcock and Troendle 1991).

Safety Considerations

No toxic effects of B_{12} have been encountered in humans or animals at any level of oral intake (Miller and Hayes 1982; IOM 1998). The overall evidence indicates that vitamin B_{12} is virtually nontoxic. Doses of 1,000 µg per day were administered to a child by intravenous injection for a year without adverse effect (Merck Service Bulletin 1958). Even if 100 percent metabolic liberation of cobalt from cyanocobalamin is assumed, the cobalt and cyanide contributions of 1,000 µg of vitamin B_{12} are toxicologically insignificant (Hathcock and Troendle 1991). It would be easy to speculate that cobalt is virtually nontoxic because of the low percentage that is absorbed by the intestine from oral intake, but the lack of toxicity of intramuscular injections of vitamin B_{12} argues strongly that the compound is nontoxic even when it is absorbed. This could be due to limited entry of cobalt into cells.

Official Reviews

IOM (1998). The IOM concluded that "no adverse effects have been associated with excess B_{12} intake from food or supplements in healthy individuals." Consequently, this organization concluded that there was no basis for a UL value.

European Commission's Scientific Committee on Food (EC SCF 2000). Likewise, the EC SCF reviewed vitamin B_{12} and found no adverse effects for vitamin B_{12} that could be used to define a LOAEL or NOAEL. They therefore found no basis for deriving a UL value.

EVM (2003). The UK's EVM found no evidence of adverse effects of vitamin B_{12} in humans. They did find that subcutaneous or intraperitoneal injections of 1.5 to 3 mg per kg body weight (100 to 300 mg in average human adults) were acutely toxic to mice (Tsao and Myashita 1993). The report concluded that there was no basis for an SUL for oral vitamin B_{12}, but they did set a guidance level of 2,000 µg per day based on a clinical trial of Juhlin and Olsson (1997) as well as other data.

European Food Safety Authority (2009). In 2009, EFSA was commissioned by the European Commission to provide a scientific opinion on the safety of vitamin B_{12}-enriched yeast (added for nutritional purposes) and on the bioavailability of vitamin B_{12} from this source. EFSA concluded that it was not possible to assess the bioavailability of vitamin B_{12} from vitamin B_{12}-enriched yeast since neither data nor suitable supporting references were provided. This provided no additional data to support a formal risk assessment of vitamin B_{12}.

CRN Recommendations

Vitamin B_{12} has no observable adverse effects at any level of oral intake, even when consumed parenterally at 1,000 µg (1 mg) twice weekly for up to 3 years or intravenously at 1 mg per day for 1 year. The IOM observation of a lack of any adverse effects for vitamin B_{12}, combined with the extensive testing and use of oral vitamin B_{12} dosages up to 1,000 µg in pernicious anemia patients (Hathcock and Troendle 1991), suggests that high dosages of vitamin B_{12} are safe for such persons.

There was evidence of growth retardation after super-high doses of oral vitamin B_{12} in mice—equivalent to 100 to 300 mg per person per day. At these levels, the adverse effects could be due to dietary dilution of other essential nutrients. Thus, there is no basis for a LOAEL for oral intake.

There is considerable experience and clinical evidence of safety at oral intakes of 3,000 µg (3 mg) per day. Higher intakes may also be safe, and a clinical trial **(**Juhlin and Olsson 1997) confirms this at 2,000 µg per person per day. Thus, the CRN UL for supplemental vitamin B_{12} is set at 3,000 µg per day. Dietary intakes are trivial in comparison with this amount of supplemental intake.

Quantitative Summary for Vitamin B_{12}

CRN UL, supplemental intake	3,000 µg (3 mg)/day
IOM UL, total intake	Not determined
EFSA UL, total intake	Not determined
EC, supplement maximum	Not determined
EVM, guidance level, supplemental intake	2,000 µg (2 mg)/day

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Biotin

Introduction

Biotin is a B vitamin and a coenzyme for carboxylase enzymes, involved in the synthesis of fatty acids and amino acids and in gluconeogenesis. It supports the health of the skin, nerves, digestive tract, and lungs. Clinical deficiency of biotin is rare. Functional deficiency of biotin has occurred through genetic defects in the enzymes that depend on it and, more rarely, through long-term consumption of large quantities of raw egg white, which contains the biotin-binding protein avidin. Impaired biotin function has serious consequences because of the resulting damage to the enzyme systems associated with respiration (Dakshinamurti 1994).

Safety Considerations

No toxic effects of oral biotin have been reported in humans. Infants have been given injections of up to 10 mg for 6 months (Miller and Hayes 1982), and oral intakes of up to 10 mg (Select Committee on GRAS Substances [SCOGS] 1978) have not produced adverse effects, demonstrating that biotin must have an extremely low order of toxicity. Acute doses of 200 mg or intravenous doses of 20 mg have not produced adverse effects (Mock 1996).

Only marginal adverse effects are produced in animals as a result of biotin doses in the hundreds of milligrams per kilogram of body weight (Institute of Medicine [IOM] 1998; European Commission's Scientific Committee on Food [EC SCF] 2001; Expert Group on Vitamins and Minerals [EVM] 2003). In view of the absence of adverse effects in humans at even extremely high doses, these effects in animals are not relevant to the safety of supplemental biotin.

Official Reviews

IOM (1998). The IOM set an acceptable intake (AI) of biotin at 30 μg. The IOM concluded that the data on adverse effects of biotin were insufficient for a risk assessment and that an UL value could not be derived.

EC SCF (2001). The EC SCF concurred that there were no data to support a risk assessment and therefore did not set an UL value.

EVM (2003). Similarly, the UK's EVM concluded that the data from studies on humans and animals were not sufficient for the establishment of a SUL. In the absence of established toxicity at any observed intake level, the EVM identified a clinical trial (Maebashi et al. 1993) that involved oral administration of 9 mg per day of supplemental biotin without adverse effects. Given the low number of individuals studied, the EVM applied an UF of 10 to conclude that biotin supplements of 0.9 mg per day should be considered safe. Considering the likely intake from food, the EVM set a guidance level for consumption from all sources at 0.97 mg per day.

CRN Recommendations

CRN concurs with the IOM, the SCF, and the EVM that a properly defined UL cannot be set because of the absence of known adverse effects at any observed level of intake. A CRN upper limit for supplements (ULS) may be identified as the highest level of intake for which there are sufficient data to support a conclusion of safety. In the U.S., biotin supplements of 5 mg and 7.5 mg are quite common. The FDA has never given public notice of receipt of any reports of adverse effects associated with biotin. The absence of adverse effect at 9 mg of biotin per day (Maebashi et al. 1993) suggests that biotin supplements with lower amounts are likely to be safe. It also suggests that the UF of 10 by the EVM is unnecessarily restrictive.

Based on (1) the absence of adverse effects at 9 mg of supplemental biotin (recognizing that the study size was small) and (2) the absence of any adverse effect reports for biotin, even though 2.5 mg and higher products are quite common in the U.S., the CRN identifies 2.5 mg as its supplement UL.

Quantitative Summary for Biotin

CRN UL, supplemental intake	2.5 mg (2,500 µg)/day
IOM UL, total intake	Not determined
EC SCF UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	9 mg (900 µg)/day

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Pantothenic Acid

Introduction

Pantothenic acid has an essential role in the metabolism of carbohydrates, fatty acids, and amino acids; in acetyl group transfers in the biosynthesis of steroids and porphyrins; and in the acetylation of some drugs (Plesofsky-Vig 1994). The name of the vitamin is derived from the Greek words meaning "from everywhere," a term that aptly fits its widespread distribution in foods. Human deficiency of pantothenic acid is rare. Experimental deficiencies in animals produce a range of defects in growth, development, metabolism, and physiological function.

Safety Considerations

Toxicity of oral pantothenic acid is extremely low, and no cases have been reported in humans. Intakes as high as 200 mg per kg per day for animals and 10 g per day for humans have been tolerated for extended periods without adverse effects (Miller and Hayes 1982; Institute of Medicine [IOM] 1998). Although most studies relate to daily consumption of 5 to 10 mg, daily amounts as high as 10 g have been consumed orally in clinical studies for many weeks without toxic effects.

Official Reviews

IOM (1998). The IOM set an acceptable intake (AI) of pantothenic acid of 5 mg per day for adults. The IOM found no reports of adverse effects of oral pantothenic acid in humans and therefore did not establish a UL.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM likewise found no reports or clinical trial evidence of adverse effects of oral pantothenic acid or calcium pantothenate in humans. Because of this, there was no basis for a proper risk assessment and they did not set a SUL. However, they did establish a guidance level derived from data showing an absence of adverse effects at supplemental intakes of 2,000 mg per day (General Practitioner Research Group 1980). A conservative UF of 10 was selected to calculate a guidance level for a supplemental intake of 200 mg per day and a total intake of 210 mg per day accounting for up to 10 mg from foods.

European Food Safety Authority (EFSA 2006). In its evaluation of pantothenic acid, EFSA noted the lack of systematic oral dose-response intake studies and very low toxicity of pantothenic acid (consumed as calcium pantothenate or panthenol). It was

concluded that no LOAEL and NOAEL could be determined, and thus no numerical UL was established. EFSA also indicated that intakes of pantothenic acid considerably higher than current intake levels from all sources do not represent a health risk for the general population.

CRN Recommendations

There are no reports of toxicity from oral administration on which a LOAEL value could be based. The clinical trial data (General Practitioner Research Group 1980) identified by the EVM provided evidence that supplemental intakes of 2,000 mg did not produce adverse effects. The amount of available information is much smaller than desirable, but with the absence of adverse effects with daily intakes as high as 10 g, and systematic clinical experience with oral intakes of up to 1,000 mg per day (Komar 1991), 1,000 mg per day is selected as the CRN supplemental UL value.

Quantitative Summary for Pantothenic Acid

CRN UL, supplemental intake	1,000 mg/day
IOM UL, total intake	Not determined
EFSA UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level	200 mg/day supplemental; 210 mg/day total intake

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Calcium

Introduction

Calcium is a nutrient most often associated with the formation, metabolism, strength, and health of bones and teeth (Institute of Medicine [IOM] 2010). Over 99 percent of calcium in the body resides in bones and teeth as a complex calcium phosphate mineral crystal. Less obvious but equally important roles for calcium occur in the soft tissues where it mediates vascular contraction, vasodilation, muscle function, nerve transmission, intracellular signaling, and hormonal secretion functions, among others. In its structural roles, calcium has a substantial impact on presence or absence of osteoporosis. Calcium absorption and utilization may be dependent on and influenced by dietary intakes of phosphorus and vitamin D, as well as other factors such as parathyroid hormone, the peptide calcitonin, and estrogen.

The role of dietary calcium and vitamin D in reducing the risk or delaying the onset of osteoporosis is now well recognized (Food and Drug Administration [FDA] 1994). Because bone loss often accompanies the aging process, sufficient calcium intake during early adulthood increases peak bone mass, thereby reducing the risk of osteoporosis decades later (Heaney et al. 2000). Increases in calcium intake in postmenopausal women delay calcium loss from bone, thus lowering the risk of declines in bone mineral density to osteoporotic levels. Calcium intakes of 1,000 to 2,000 mg per day have been shown to increase or slow the decline in bone density and to reduce the risk of osteoporosis (FDA 1994).

Safety Considerations

A number of hypotheses for adverse effects of excess calcium intake have been investigated over the years, including kidney stones (nephrolithiasis) (Johnson et al. 1979), hypercalcemia with renal insufficiency (milk-alkali syndrome) (Junor and Catto 1976; Orwoll 1982), and harmful calcium interactions with other minerals (Spencer et al. 1965; Clarkson et al. 1967; Schiller et al., 1989). The evidence regarding a link to an increased risk of kidney stones with high calcium intake from foods and supplements is inconsistent, with some studies associating higher calcium intakes with *decreased* risk of kidney stones (Curhan et al. 1993). High dietary calcium levels can influence the bioavailability and absorption of many trace elements—particularly the divalent cations, such as magnesium, manganese, and zinc—but it is unlikely that these effects are commonly severe enough to have clinical impact (Greger 1988). The intestinal interactions have been studied primarily in animals.

Official Reviews

IOM (1997, 2010). The IOM evaluated the various potential adverse effects of excess calcium intake and concluded that kidney stone formation was the only one with appropriate data to support a risk assessment (IOM 1997). The IOM identified the following tolerable upper intake level (UL) values for calcium: 2,500 mg for children up through 8 years of age, 3,000 mg for those ages 9 through 18 years, 2,500 mg for adults ages 19 through 50 years, and 2,000 mg for adults ages 70 years and older. There are some difficulties with these values, however, since the UL is based on a UF that varies from one example to another. The IOM recognized that the data from patients with kidney stones were not likely to be meaningful for normal adults and thus did not utilize the data of Burtis et al. (1994), which might have indicated a LOAEL of 1,685 mg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM published its findings on calcium in 2003. Concluding that the available data were insufficient to set a safe UL, they instead determined a guidance level of calcium intake at which milk-alkali syndrome, constipation, and bloating would be avoided. The report recognized that few side effects have occurred in clinical trials with 1,600 or 2,000 mg of supplemental calcium (Levine et al. 1997; Hofstad et al. 1998; Bonithon-Kopp et al. 2000). Based on a mean dietary calcium intake of 830 mg per day in the UK, the EVM set the guidance level for supplemental calcium at 1,500 mg per day, stating that such a supplemental level "would not be expected to result in any adverse effect."

EFSA (2012). EFSA considered a number of human intervention studies in adults. These studies showed a lack of adverse effects associated with daily calcium intakes of 2,500 mg from both diet and supplements. Taken together with the robust database used by the European Commission's Scientific Committee on Food (EC SCF) to establish a UL of 2,500 mg in 2003 (based on a NOAEL of 2,500 mg and a UF of 1), EFSA also proposed a UL of 2,500 mg per day of calcium from all sources for adults.

Recent Concerns

Published results from numerous epidemiological studies and one meta-analysis of select randomized controlled clinical trials have prompted concern about possible associations between calcium use and a small increase in risk of adverse cardiovascular events. Because of significant limitations in design or interpretation, these reports do not provide strong evidence of harmful cardiovascular effects of calcium supplementation.

A subgroup analysis from a large clinical trial—the Women's Health Initiative (WHI)—played an important role in the meta-analysis. A number of limitations in the design and execution of this trial invalidate many generalizations based on it. These limitations include (1) inadequate monitoring and assessment of compliance with the treatment protocol, (2) use of nontrial calcium supplements by the majority of subjects in the placebo and calcium treatment groups, and (3) lack of information on and adjustment for known cardiovascular risk factors. With these limitations, confounding and bias cannot be excluded as explanations for the results (Bolland et al. 2011).

No suggestions of serious adverse effects from calcium supplements or calcium with vitamin D had been reported until Bolland, Reid, and coworkers raised the issue of possible increased risk of adverse cardiovascular events (Bolland et al. 2008; Reid et al. 2008). Although some of the data suggested a hazard ratio for calcium or calcium plus vitamin D as high as 1.43 (43 percent increase in risk), after adjustment for known cardiovascular risk factors, statistical significance was lost (Bolland et al. 2008).

Bolland, Reid, and colleagues followed these research articles with a meta-analysis from other clinical trials (Bolland et al. 2010), as well as the subgroup analysis from the WHI (Bolland et al. 2011). On the basis of this and a follow-up meta-analysis, these researchers concluded that calcium supplementation, with or without vitamin D, modestly increases the risk for myocardial infarction or stroke and recommended that the use of such supplements in older people should be reassessed (Bolland et al. 2011).

However, the conclusions and recommendations of Bolland, Reid, and colleagues, based on their own data and interpretations, have been questioned by a number of experts who have raised concerns and unanswered questions about the methodology employed and the potential for bias and confounding (Letters to the Editor 2008, 2010, 2011; Bockman et al. 2011; Nordin et al. 2011). These concerns remain unanswered.

More recently, Li and colleagues reported that, in a large epidemiological study, higher intakes of total dietary and dairy calcium significantly reduced the risk of myocardial infarction but users of calcium supplements had significantly increased risk (Li et al. 2012).

Given the widespread use of calcium supplements and the potential of harm from inadequate calcium intake, the Council for Responsible Nutrition (CRN) concluded that a thorough examination of the evidence for harm and for benefit from calcium supplementation was warranted. To accomplish this goal, CRN convened a group of

academic and industry experts to develop a consensus on the available evidence, with emphasis on five of the Bradford-Hill criteria for causal inference from data: strength, consistency, dose-response, biological plausibility, and results from experimentation.

Heaney and colleagues summarized data not only from the papers by Bolland et al. and Li et al. but also results from other pertinent long-term prospective cohort studies and clinical trials (Heaney et al. 2012). A review and meta-analysis by Wang et al. (2010), funded by the American Heart Association and the National Heart, Lung, and Blood Institute, showed that the relative risk for cardiovascular disease events was 1.14 (95 percent confidence interval, 0.92 to 1.41) in studies involving calcium supplementation without vitamin D. An additional meta-analysis including clinical trials with both calcium and vitamin D showed a relative risk of 1.04. The authors concluded that vitamin D at moderate to high intakes may reduce cardiovascular disease risk, whereas supplementation with calcium alone seems to have minimal cardiovascular effects.

Although there was no overall indication of a connection between calcium intake and atherosclerotic heart disease or stroke, a few of the cited studies showed a weak but statistically significant positive association of calcium intake and cardiovascular disease, whereas a similar number show the opposite (protective) effects. Because of these mixed results, Heaney and colleagues determined that the findings from available clinical trials and prospective cohort studies indicate that there is no significant effect of calcium supplements on cardiovascular disease (Heaney et al. 2012).

CRN Recommendations

A wide range of clinical and epidemiological studies discussed by the IOM, the EC SCF, the EVM and several published reviews and meta-analyses have shown no adverse effects with calcium intakes of 2,000 mg or less in adults ages 51 years or older. Based on the judgment of the IOM, the calcium UL for persons aged 19 through 50 years should be 2,500 mg, which is the midpoint between the value for individuals ages 51 years and older and the 3,000 mg UL for adolescents. Considering the quite variable calcium intake from foods, dairy products, and fortified foods, CRN agrees with the EVM that a maximum supplement level for adults should be 1,500 mg. Thus, the CRN UL for supplemental for calcium is set at 1,500 mg per day for adults.

Quantitative Summary for Calcium

CRN UL, supplemental intake	1,500 mg/day for most adults
IOM UL, total intake	3,000 mg/day for adolescents; 2,500 mg/day for adults ages 19–50; and 2,000 mg/day for adults ages 50 and older
EFSA UL, total intake	2,500 mg/day
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	1,500 mg/day

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Phosphorus

Introduction

Phosphorus in the phosphate form is an essential nutrient involved in many physiological processes, such as the body's energy cycle and regulation of acid-base balance. It is a component of cell membranes (as part of phospholipids); in cell regulation and signaling; and in the mineralization of bones and teeth (as part of hydroxyapatite). High-energy phosphate bonds are involved in the structure of the genetic materials DNA and RNA, and phosphorus helps the body make ATP, a molecule the body uses to store energy (Arnaud and Sanchez 1996; Knochel 1999).

The dietary requirement for phosphorus is based on the maintenance of normal serum phosphate levels in adults, which was also believed by the Institute of Medicine (IOM) to provide adequate intake to meet cellular and bone formation needs (IOM 1997). Phosphorus is widespread in the food supply, and dietary phosphorus deficiency is usually seen only in cases of anorexia or frank starvation. Phosphorus deficiency can result from the excessive use of antacids that contain aluminum hydroxide, which precipitates dietary phosphorus as insoluble and unabsorbable aluminum phosphate in the intestine (IOM 1997). While it is well established that phosphate and calcium are required for bone formation, emerging evidence suggests that phosphate supplementation may play a role in the effectiveness of calcium in reducing the risk of developing osteoporosis (Heaney and Nordin 2002; Heaney and Weaver 2003; Shapiro and Heaney 2003).

Safety Considerations

The phosphorus level in normal diets is not harmful, especially given adequate intakes of calcium and vitamin D (IOM 1997). Most dietary supplements do not contain significant amounts of phosphorus, and the contribution of dietary supplements to phosphorous intake is low (Bailey et al. 2011). A calcium-to-phosphorus ratio lower than 1 to 2 can cause small decreases in blood calcium levels; therefore, a ratio closer to 1 to 1 is considered superior. Phosphorus requirements are influenced by interactions between calcium and phosphate, but studies have demonstrated that significant changes in phosphorous intake may not affect calcium balance in a meaningful way. For example, an increase in dietary phosphorus from 800 to 2,000 mg per day in adult males did not affect calcium balance regardless of calcium intake (Arnaud and Sanchez 1996).

In the absence of clinical signs of excess phosphorus, plasma phosphorus level is the most reliable indicator of excess phosphate (IOM 1997). There is no convincing scientific support for the widely accepted notion that consuming too much phosphorus from certain carbonated beverages contributes to calcium loss and increases the risk of osteoporosis (Heaney 2002). Indeed, the opposite effect may be true—calcium intake without simultaneous phosphorus intake may decrease the utilization of the calcium, at least partly neutralizing the potential benefits of the calcium on bone renewal.

Official Reviews

IOM (1997). The IOM reviewed dietary phosphorus for potential adverse effects, including adjustment in calcium-regulating hormones, metastatic calcification, skeletal porosity, and interference with calcium absorption, and found no evidence of any such activity (IOM 1997). In the absence of overt adverse effects, the IOM selected plasma phosphorus levels as the appropriate indicator of excess phosphorus intake. Therefore the IOM based its estimates of safe upper intake levels on the levels necessary to derange plasma phosphorus homeostasis. The IOM identified a NOAEL of 10.2 g per day and applied a UF of 2.5 to derive a UL of 4 g per day for adults. The UF of 2.5 is a default value meant to account for the uncertainty related to the pharmacokinetic relationship between food intake and blood levels (Petley et al. 1995). The IOM report indicates that phosphorus intakes may have increased dramatically over the last decade, thus creating concern about excessive phosphorus. This suggestion is counterbalanced by the evidence that calcium supplements can induce temporary low values in plasma phosphorus and that cosupplementation with calcium and phosphate can nullify this effect (Heaney 2002; Heaney and Nordin 2002).

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM, like EFSA, found the existing evidence to be insufficient to derive an SUL value. It instead set a guidance level for total and supplemental phosphorus. The organization expressed concern about the few reports of mild gastrointestinal symptoms, such as osmotic diarrhea and gastrointestinal disturbance, reported in relation to supplemental phosphorus above 750 mg per day; on this basis it identified a NOAEL of 750 mg supplemental phosphorus per day. A guidance level of 250 mg supplemental phosphorus was identified by applying a default UF of 3 to this NOAEL. Assuming an intake of 2,100 mg from food and water, the EVM concluded that a guidance level of 2,400 mg was appropriate for phosphorus total intake from all sources.

European Food Safety Authority (EFSA 2006). In 2006 EFSA published its scientific opinion on the safe upper levels of phosphorous. The EFSA-appointed Scientific Panel

on Dietetic Products, Nutrition and Allergies concluded that the available data are not sufficient to establish an upper level for phosphorus. The available data indicate that normal healthy individuals can tolerate phosphorus (phosphate) intakes up to at least 3,000 mg per day without adverse systemic effects. In some individuals, however, mild gastrointestinal symptoms have been reported if exposed to supplemental intakes above 750 mg phosphorus per day. There is no evidence of adverse effects associated with the current dietary intakes of phosphorus in EU countries.

CRN Recommendations

In adults with normal kidney function, phosphorus is readily excreted, and no imbalance in calcium metabolism occurs except at extreme intakes (Arnaud and Sanchez 1996). There are no data appropriate for identifying direct adverse effects of dietary phosphorus, and therefore no LOAEL can be identified. Similarly, no specific intake level qualifies as the NOAEL level. The very high NOAEL value identified by the IOM is perhaps too hypothetical, just as the very low NOAEL identified by the EVM was based on a speculative, worst-case interpretation of a very few reports of gastrointestinal side effects that could have had other causes. There is a need for an appropriate ratio of calcium-to-phosphorus intake within a broad range of acceptable ratios; therefore, in the absence of more specific evidence, a CRN UL of 1,500 mg is set for supplemental phosphorus.

Quantitative Summary for Phosphorus

CRN UL, supplemental intake	1,500 mg/day
IOM UL, total intake	4,000 mg/day
EFSA UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level	250 mg/day supplement; 2,400 mg/day total intake

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Magnesium

Introduction

Magnesium plays a vital role in a wide range of biochemical and physiological processes, particularly those involving energy metabolism and utilization as well as bone structure (Institute of Medicine [IOM] 1997; Shils 1999). Clinical consequences of magnesium deficiency include a variety of neurological and neuromuscular signs such as tremors, spasms, and altered reflexes. In addition, magnesium deficiency may cause or exacerbate myocardial infarction, arrhythmia, and associated mortality. It is often brought on not only by dietary inadequacy but also by malabsorption; kidney dysfunction, often as increased excretion under the influence of diuretic drugs (National Institutes of Health [NIH] 2013); endocrine disorders; genetic and congenital disorders; and alcoholism (Shils 1996; NIH 2013). Magnesium is efficiently absorbed in the intestine, and body concentrations are controlled primarily through the regulation of urinary excretion rates. It is stored and reserved in the skeleton (Shils 1994, 1999; IOM 1997).

Safety Considerations

Healthy human kidneys are capable of rapidly excreting large amounts of absorbed or injected magnesium. Even after large intakes, serum levels usually stay within the usual and safe range (IOM 1997; Shils 1999). Subjects with normal kidneys can excrete 40 to 60 g of magnesium per day without side effects when the mineral is administered via persistent infusion. Elevated serum levels may occur when drugs that contain magnesium, usually antacids and cathartics, are taken in excess of 15 g per day on a chronic basis (Smilkstein et al. 1988). Moderate increases in plasma magnesium levels may induce symptoms such as nausea, vomiting, and hypotension (Shils 1996, 1999; NIH 2013). Due to the major involvement of magnesium in neurological functions, the elevated plasma levels that occur as a result of large intravenous infusions can cause adverse effects to become more severe and sometimes life-threatening.

Adverse effects of magnesium are primarily related to three conditions: neonatal neural depression after intravenous maternal treatment for eclampsia, accidental or deliberate poisoning with very large single doses, and increased sensitivity to magnesium-containing drugs in people with renal failure (Flink 1976). Aside from osmotic diarrhea related to unabsorbed magnesium, there is no evidence that large quantities of oral magnesium are harmful to people with normal kidney function (IOM 1997).

Average total dietary intakes of magnesium by U.S. adults is 300 to 400 mg per day (IOM 1997; Hunt and Johnson 2006). Supplemental intakes of 375 mg lack any known adverse effects (Stendig-Lindberg et al. 1993), and it is not until supplements reach levels greater than 10 mg per kg per day (700 mg in a 70-kg person) that plasma magnesium concentrations become elevated (Durlach et al. 1994).

Possible negative consequences of calcium's interaction with magnesium have been hypothesized but have not been reported.

Official Reviews

IOM (1997). The IOM concluded that the magnesium found in foods has not been found to produce adverse effects and that "the primary initial manifestation of excessive magnesium intake from other oral nonfood sources is diarrhea." The physiological effects of longer-term high intakes of oral magnesium have been observed only in persons with abnormal kidney function. Thus, the critical adverse effect identified as the appropriate basis for the magnesium UL is diarrhea. In its dose-response evaluation, the IOM identified a few studies, mainly in the frail elderly, that found some increase in the incidence of diarrhea with supplemental intakes of magnesium chloride or other soluble salts in the range of 360 to 460 mg of magnesium per day (Marken et al. 1989; Ricci et al. 1991; Bashir et al. 1993), but noted that foods enriched with 452 mg of magnesium as magnesium oxide did not cause diarrhea (Altura et al. 1994). Another study (Stendig-Lindberg et al. 1993) found no diarrhea in postmenopausal women who were given up to 678 mg magnesium as magnesium hydroxide. Similarly, diabetic subjects supplemented with 400 mg magnesium as an oxide or chloride experienced no diarrhea (Nadler et al. 1992). Elderly subjects given 372 mg of magnesium did not have any increase in diarrhea or gastrointestinal complaints (Paolisso et al. 1992). On the basis of these studies, in particular that of Bashir and coworkers, the IOM identified a LOAEL of 360 mg for nonfood magnesium. To derive the UL, the IOM selected a UF of 1.0, even though it was being applied to a LOAEL, because of "the very mild, reversible nature of osmotic diarrhea caused by ingestion of magnesium salts." The relative adverse effects of the different chemical compounds of magnesium have not been systemically studied.

European Commission, Scientific Committee on Food (EC SCF 2001). The EC SCF agreed that osmotic diarrhea is the critical effect for identification of a UL for magnesium. It identified a LOAEL of 360 mg and a NOAEL of 250 mg per day for nonfood magnesium. Selecting a UF of 1.0 for application to the 250 mg NOAEL, the SCF derived a UL of 250 mg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM concurred that osmotic diarrhea is the adverse effect of concern, but determined that the data are insufficient to establish an SUL value. Instead, they established a guidance level of 400 mg per day for nonfood magnesium because "it would not be expected to result in any significant adverse effects."

All three reviews found no evidence that magnesium intake from food causes osmotic diarrhea but that nonfood sources such as supplements, laxatives, and antacids have the potential to produce these mild, reversible adverse effects. Thus, the SUL or guidance values identified were applied to nonfood sources only.

CRN Recommendations

The only severe adverse effects reliably attributed to oral consumption of magnesium relate to prolonged use in multiple-gram quantities as an antacid or cathartic. Mild to moderate but infrequent and easily reversible diarrhea can result from nonfood magnesium intakes at levels above 400 mg per day. The mild nature of this adverse effect makes a LOAEL unnecessary and suggests that a UF of 1.0 is appropriate for deriving a UL for supplements. Thus, the CRN UL for supplemental magnesium is 400 mg per day for healthy adults. Multiple doses separated by several hours is preferred in order to further dilute any adverse effects. Individuals consuming supplements should be aware that some antacids and laxatives also contain magnesium.

Quantitative Summary for Magnesium

CRN UL, supplemental intake	400 mg/day
IOM UL, nonfood sources	350 mg/day
EC SCF UL, nonfood sources	250 mg/day
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	400 mg/day

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Potassium

Introduction

Potassium is an essential element required for a large number of physiological electrolyte and osmolar regulations (Oh and Uribarri 1999; Institute of Medicine [IOM] 2004). Potassium is widely available in food, and deficiency is more likely to be brought on by impaired renal function than by insufficient intake (McLaren 1999; IOM 2004). Manifestations of potassium deficiency include muscle weakness, respiratory inadequacy, hypotension, and electrocardiographic abnormalities. Similarly, potassium toxicity is more likely to result from renal insufficiency (due to decreased kidney function or decreased water intake) than from excess consumption (McLaren 1999).

Safety Considerations

Normal serum potassium levels are between 3.5 and 5.0 mEq per L. The risk of exceeding this level through normal dietary or supplemental intake of potassium is small in healthy adults. Cases of hyperkalemia (toxic levels of potassium in the blood, exceeding 6.5 mEq per L) are usually the result of renal failure or disorders such as Addison disease. Hyperkalemia can result in serious cardiac toxicity, but the amounts of potassium associated with such hyperkalemic states depend heavily on water consumption and kidney function. Because of the impact of these factors as well as that of other electrolytes (principally sodium and chloride), the evidence for potassium safety or toxicity at any particular intake level must be judged cautiously.

Several trials have evaluated the effects of potassium supplementation. Siani et al. (1991) found no adverse effects of potassium chloride at daily doses of 1,900 mg. Fotherby and Potter (1992) found no adverse effects at 2,340 mg per day. However, the evaluations for possible adverse effects (Expert Group on Vitamins and Minerals [EVM] 2003) were not specified endpoints in these clinical trials.

Potassium doses of 1,250 mg administered 3 times per day (for a daily total of 3,750 mg) produced only minor and infrequent adverse effects as revealed by endoscopy (McMahon et al. 1982). In a follow-up study, the wax-matrix formulation was administered in dosages ranging from 900 to 3,700 mg per day (McMahon et al. 1984). Endoscopically evident erosions of the upper GI tract were evident in a few subjects supplemented with 1,560 to 3,120 mg potassium per day for 21 months. Gastrointestinal symptoms were mild and did not correlate with lesions shown by endoscopic evaluation.

A meta-analysis of clinical trials on potassium (mostly potassium chloride) for possible lowering of blood pressure indicated that this mineral "appeared to be well tolerated in all studies included" (Whelton et al. 1997). The potassium dosages in those clinical trials ranged from 1,876 to 7,820 mg per day. The dietary potassium levels were not identified, but are usually in the 2 to 5 g range.

Official Reviews

Food and Drug Administration (FDA 1975). The FDA in 1975 issued a statement that "there have been several reports, published and unpublished, concerning nonspecific small-bowel lesions" related to use of oral drug products containing 100 mg or more potassium. It subsequently required precautionary labeling of such products. The FDA did not provide any dose-response evaluation that would justify such a finding, but concluded that any capsule or coated tablet of a potassium salt intended for oral ingestion without prior dilution with an adequate volume of liquid to preclude gastrointestinal injury should carry the FDA prescribed warning statement.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM 2003 review concluded that the evidence was not sufficient to set an SUL for potassium but could support a guidance level. From the clinical trial evidence judged to be most relevant (McMahon et al. 1982; McMahon et al. 1984; Grimm et al. 1988, 1990), the EVM concluded that "supplemental doses of up to 3,700 mg potassium per day appear to be without overt adverse effects, but may be associated with gastrointestinal lesions diagnosed by endoscopy." Based on this conclusion (with no correction for uncertainty), the EVM set 3,700 mg per day as the guidance level. It was not specified whether this guidance level applied to supplemental potassium or total intake forms all sources. The EVM recognized that the recommended nutrient intake (RNI) in the UK for potassium was 3,500 mg for adults over 18 years of age, but did not identify any estimate of average potassium intake by the population as a whole.

IOM (2004). The IOM has reviewed potassium, the other electrolytes, and water to establish new dietary reference intakes (DRIs). The IOM concluded that there was no evidence of chronic excess intakes of potassium in apparently healthy individuals and thus no UL was established.

European Food Safety Authority (EFSA 2005). EFSA concluded that the available data were insufficient to establish a UL for potassium, but noted that potassium intakes from foods in healthy individuals (average 3 to 4 g per day in adults, generally not exceeding 5 to 6 g per day), as well as supplemental potassium as potassium chloride of

about 3 g per day, have not been associated with adverse effects. EFSA noted that certain groups are sensitive to increases in potassium intakes, in particular those with impaired renal excretion of potassium.

European Food Safety Authority (EFSA 2010). EFSA also published reviews of potassium and sodium sulfate safety in 2010. The EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) was asked by the European Commission to deliver a scientific opinion on the safety of potassium sulfate and of sodium sulfate when added for nutritional purposes in food supplements as sources of, respectively, potassium and sodium. The review was limited to a review, per the petitioners' request, to review potassium sulfate used in food supplements to provide a maximum of 100 mg potassium per day for adults. EFSA concluded that the proposed use and use levels of potassium sulfate as a sources of potassium were not a safety concern.

CRN Recommendations

The clinical trial data on potassium chloride, together with the epidemiology supporting the safety of larger amounts of potassium from fruits and vegetables, indicate that this nutrient has a wide margin of safety. Clinical trials collectively show no pattern of adverse effects for supplemental potassium of 1,500 mg, with the potassium from food being unspecified. Larger quantities of potassium as potassium chloride can produce gastrointestinal effects, and these seem more likely if the daily total is ingested all at once, especially on an empty stomach. The EVM established a guidance level of 3,750 mg but did not distinguish between food intake and supplement intake. The evidence that was used in the EVM's determination, however, related only to supplemental potassium.

Considering clinical trial evidence and the apparent safety of potassium intakes as high as 8 to 11 g per day from fruits and vegetables, CRN sets its ULS for potassium at 1,500 mg per day, with the provision that it should be divided into doses no larger than 500 mg each. There is no discernible scientific justification for the FDA threshold of 100 mg of potassium for regulation of products such as drugs that require a prescription caution statement.

Quantitative Summary for Potassium

CRN UL, supplemental intake	1,500 mg/day (500 mg, 3 times a day)
IOM UL, total intake	Not determined
EFSA UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	3,700 mg/day

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Boron

Introduction

Boron is essential to the life cycles of some animal species, but in regard to humans is considered only *probably* essential. There is, however, clear evidence that dietary intakes of this element are beneficial to humans (Nielsen 2000). In humans, boron deprivation impairs calcium metabolism and bone health as well as brain function and energy metabolism.

Studies of dietary deprivation on boron in animals have reported adverse effects (e.g., on growth, serum steroid hormone concentrations, and bone calcification) that can be corrected by increasing boron intake. The effects of low boron intakes are more marked when accompanied by low status for other nutrients (e.g., vitamin D and magnesium) (European Food Safety Authority [EFSA] 2006).

Boron occurs in foods as borate and boric acid.

Safety Considerations

Boron has a low potential for causing obvious adverse effects in adult humans, as indicated by the widespread use of boric acid between 1870 and 1920 as a food preservative. This use of boric acid led to boron intakes of up to 500 mg per day without adverse effects other than nausea and loss of appetite (Nielsen 1996). Intakes of 500 mg boric acid (72 mg boron) per day for 50 days by adults have disturbed appetite and digestion (Nielsen 1996).

In short- and long-term animal studies, oral exposure to boron at levels greater than 13 mg per kg per day have resulted in various adverse effects. Reproductive and developmental toxicity were the most critical adverse effects reported in these studies. In pregnant rats, dietary boric acid (13 mg boron per kg and higher) can cause fetal development defects and growth deficits (Price et al. 1996). In studies with dogs, high intakes of boric acid (29 mg boron per kg per day) have caused testicular atrophy and moderately decreased sperm production (Weir and Fisher 1972). No evidence of carcinogenicity has been reported in long-term animal studies, and the available data indicate that boric acid is not genotoxic.

To calculate a safe intake (i.e., a reference dose, or RfD), the U.S. Environmental Protection Agency (EPA) relied on the dog study reported in Weir and Fisher (1972). In this study, adverse effects were found with an intake of 29 mg per kg per day over 38 weeks of treatment, and this level became the LOAEL. The next lower dose, of 8.8 mg

per kg per day, produced no adverse effects; this intake level became the NOAEL. The EPA applied a hundredfold margin of safety to the NOAEL in dogs to calculate an RfD of 0.09 mg per kg per day, or 6.3 mg per day in a 70-kg human (EPA 2004).

For humans, the data are too scant and the effects too vague to identify a specific LOAEL value. Although more information is needed, the gastrointestinal effects associated with intake of 500 mg of boric acid (72 mg boron) may be considered undesirable rather than harmful. Moreover, they should be self-limiting due to consumer awareness. Thus, EPA could not propose a LOAEL value for boron intake by humans (EPA 2004).

Clinical trials with an upper intake of 3 mg per day produced no adverse effects (Meacham et al. 1994; Nielsen 2000). However, because so few other intake levels have been subjected to clinical study, 3 mg may be lower than appropriate to identify as a NOAEL for humans. The EPA value of 0.09 mg per kg per day, or 6.3 mg per day in a 70-kg man, may be considered a safe level of human intake. This intake level cannot be identified as a NOAEL, however, because it is based on calculation rather than observation.

Official Reviews

Institute of Medicine (IOM 2001). The IOM found that most data on the adverse effects of boron in humans were associated with an accidental single episode or short-term ingestion of boric acid. In the absence of human dose-response data judged useful, the IOM extrapolated from animal data to set a human UL. From the data of Price and coworkers (Price et al. 1996), the IOM identified a NOAEL of 9.6 mg per kg per day for developmental toxicity in mice, and it selected a composite UF of 30 (3 for interspecies variability and 10 for extrapolation from mice to humans) to derive an UL of 0.3 mg per kg per day. Correction to a reference adult weight of 61 kg gave the IOM an adult UL of 20 mg boron per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM found the long-term clinical study of Meacham and colleagues (Meacham et al. 1994) to be an insufficient basis for an SUL or guidance level. Instead, using the same data on developmental toxicity in mice (Price et al. 1996) that the IOM studied, the EVM identified the NOAEL as 9.6 mg per kg per day and applied a composite UF of 60 (10 for interspecies variability and 6 for interindividual variability). This resulted in a SUL of 9.6 mg per day for a 60-kg person.

EFSA (2004). EFSA reviewed the safety of boron and established an UL of 10 mg per person per day for adults. This value is based on the NOAEL of 9.6 mg per kg per day for decreased fetal body weight in rats following in utero exposure (Price et al. 1996) and extrapolated to humans by applying a UF of 60.

CRN Recommendations

A clinical trial (Meacham et al. 1994) with an intake of 3 mg per day produced no adverse effects. Other studies confirmed this observation (Nielsen 2000), and that intake may be considered the highest observed intake (HOI) for supplementation. Intakes from conventional foods are almost always less than 3 mg per day (EVM 2003).

For boron intakes by adults, the EPA value of 6.3 mg per day may used as a well-substantiated human NOAEL; that is, it does not require application of any additional safety factor (in other words, a safety factor of 1.0 is sufficient) to calculate a safe human intake.

In the face of these quantitative uncertainties, CRN recommends a supplemental UL of 6 mg per day, based on the EVM UL of 9.6 mg and the fact that food intakes rarely exceed 3 mg.

Quantitative Summary for Boron

CRN UL, supplemental intake	6 mg/day
IOM UL, total intake	20 mg/day
EFSA UL, total intake	10 mg/day
EC supplement maximum	Not determined
EVM SUL, total intake	9.6 mg/day

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Chromium

Introduction

The biological effects of chromium strongly depend on its specific chemical form. Nutritive effects are exclusively related to chromium III (valence 3+), and all major toxic effects are associated with chromium VI (valence 6+) (Nielsen 1994; Environmental Protection Agency [EPA] 1998). The first recognized nutritional effects of chromium were its actions as a "glucose tolerance factor," a function that relies on the ability of trivalent chromium (III) to potentiate the action of insulin with chromium as a part of one or more organic complexes (Nielsen 1994; Stoecker 1999). Some researchers have reported that chromium may influence body composition in animals (Page et al. 1993) and humans (Bulbulian et al. 1996), but other research did not find such effects (Lukaski et al. 1996). Recent studies have found that chromium supplementation, in the form of chromium picolinate, decreased symptoms in type II diabetes patients and that 1,000 μg per day of chromium in this form were more effective to that end than 200 μg per day in other forms (Anderson, Bryden, et al. 1997). This beneficial effect of chromium picolinate has been attributed to increased insulin action rather than to increased secretion (Ghosh et al. 2002). The usual dietary intakes of chromium are 25 to 45 μg (Institute of Medicine [IOM] 2001, 2006).

Safety Considerations

Chromium VI (chromate, valence 6+) is clearly established as the work-related etiological agent in lung disease, including lung cancer, in chromate and stainless steel workers (Gad 1989). However, this form (chromium VI) is not produced from dietary forms by any biological system and thus data on it are not relevant to dietary chromium safety.

Regarding chromium III (valence 3+), no credible data or reports have shown adverse effects in humans from its consumption, and animal data also suggest that orally administered chromium is extremely innocuous (Dourson 1994; Nielsen 1994; Hathcock 1996; IOM 2001; Expert Group on Vitamins and Minerals [EVM] 2003; European Food Safety Authority [EFSA] 2010).

The potential genotoxicity of chromium III has been assessed in several experiments. Results of in vitro assays are conflicting, with some evidence indicating that certain chromium III compounds may cause chromosomal damage in vitro at high concentrations (EFSA 2010); however, chromium III compounds do not have genotoxic activity in vivo

(EFSA 2010). Moreover, ingestion of various chromium III compounds did not produce carcinogenicity in mice and rats in long-term studies (Schroeder et al. 1964, 1965; Ivankovic and Preussman 1975). The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services (DHHS) performed carcinogenicity studies on chromium picolinate monohydrate and concluded that there was no evidence of carcinogenic activity due to the tested substance in female rats or in male or female mice (DHHS 2008; Stout et al. 2009). Although the NTP stated that there was equivocal evidence of carcinogenic activity in male rats (based on an increase in the incidence of preputial gland adenomas), the absence of a dose-response effect, as well as the lack of such effects across sexes or species, indicates that chromium picolinate is not carcinogenic.

The available data from studies in mice provide evidence of a lack of developmental toxicity associated with chromium III ingestion. In a limited number of animal studies, inconsistent findings with respect to reproductive toxicity have been reported, with no effects on reproduction parameters observed in some studies but some effects noted in others (EFSA 2010). The LOAEL values from the latter studies are several orders of magnitude higher than the intake of chromium III from food and supplements, indicating a large margin of safety.

Picolinic acid is a metabolite of tryptophan, and total daily exposure via this route is many times higher than the amounts contained in chromium picolinate dietary supplements. It is naturally present in human breast milk (3 μM), cow milk (5 μM), and other foods such as broccoli, beans, and potatoes (Robello et al. 1982). The estimated urinary output of picolinate by adults is 14 mg per day (Evans 1993). A chromium picolinate-containing dietary supplement ($CrPic_3$) with 200 μg chromium would include 1.4 mg of picolinate. Assuming total absorption of the picolinate from this strength supplement and no catabolism of the picolinate, the supplement would increase urinary output by 10 percent. Even with a chromium-containing supplement level of 1,000 μg, the daily picolinate exposure of adults would be increased by only 50 percent.

Human clinical trials have provided strong support for the safety of chromium supplements in chromium picolinate form at levels of up to 1,000 μg per day (IOM 2001, 2006; Broadhurst and Domenico 2006). No pattern of adverse effects has been observed in these trials.

A number of anecdotal reports have attributed adverse effects to supplements of chromium in general (IOM 2001; EVM 2003) and to chromium picolinate in particular (Wasser et al. 1997). One report, concerning a single case of renal failure in a person who

was taking chromium picolinate, attributed the disease to the chromium supplement (Wasser et al. 1997). The authors acknowledged that the patient also received "antihypertensive agents," but nonetheless attributed the effect solely to chromium picolinate; the renal toxicity of antihypertensive agents and pathological effects of poorly controlled hypertension apparently were not considered. Critical letters were published in response to the report (Michenfelder et al. 1997; Hathcock 1997), pointing out the flaws in the conclusion. Despite these methodological issues and the critical response, the case is often cited without caveat (Hepburn et al. 2003; IOM 2006).

Official Reviews

World Health Organization (WHO 1996). The WHO reviewed the safety of chromium supplementation and considered that supplementation with chromium should not exceed 250 µg per day. It was noted, however, that research suggests that the upper limit of the safe range of population mean intakes of chromium could be above this level.

EPA (1998). From chromic oxide data with mice, the EPA identified 1.47 g of chromium per kg of body weight as the NOAEL in animals but could not identify a LOAEL. By rounding down to 1 g per kg and applying a composite UF of 1,000, the EPA calculated a chromium maximum of 1 mg per kg, which is equivalent to 70,000 µg for a 70-kg person. Thus, chromium III has an extraordinarily wide margin of safety.

IOM (2001). The IOM could not identify a mean requirement, but set its AI at 25 µg for young adult women and 35 µg for young adult men. The adequacy of intakes in this range is supported by normal dietary chromium consumption in healthy persons, but it is not clear that such intakes lower the risk of type II diabetes in middle age. The IOM considered the evidence related to chronic renal failure, genotoxicity, carcinogenicity, hepatotoxicity, reproductive toxicity, and other possible effects and could not identify a hazard or dose-response relationship for soluble salts of dietary chromium (that is, chromium III). Thus, the IOM did not set a UL. The organization has released a draft monograph on the safety of chromium picolinate, but no risk assessment conclusion was reached for this form of chromium (IOM 2006). The draft report did, however, supply an excellent summary of the clinical trials that have been done on this ingredient.

European Commission, Scientific Committee on Food (EC SCF 2003). The EC SCF reviewed chromium toxicity and reached conclusions that were, as a whole, the same as those reached by the IOM. The animal data of Anderson, Cheng, et al. (1997) were considered, but, given the absence of adverse effects, the EC SCF decided not to set an UL for chromium.

EVM (2003). The UK's EVM found no credible evidence of adverse effects but identified a guidance level of 10,000 μg (10 mg) per day, based on extrapolation from animal research on chromium chloride and chromium picolinate. In making this decision, the EVM derived its guidance level directly from the experiments of Anderson and coworkers, who performed histopathological examinations of the treated rats and found no adverse effects resulting from chromium chloride ($CrCl_3$) or chromium picolinate ($CrPic_3$) (the rats were fed 15 mg of chromium per kg of body weight per day) (Anderson, Cheng, et al. 1997). A composite UF of 100 was applied by the EVM to the highest level of chromium chloride used, which was identified as the NOAEL. In the Anderson study relied upon by the EVM, chromium chloride and chromium picolinate were used with the same levels of chromium, and each produced no evidence of toxicity. The EVM refused to apply the guidance level derived from Anderson's data to the picolinate form based on findings of DNA damage caused by chromium picolinate to mammalian cells in vitro. Subsequently, the Committee on Mutagenicity reviewed further data and concluded that the balance of the evidence suggested that chromium picolinate was not genotoxic. Accordingly, the UK Food Standards Agency (FSA) stated in 2004 that there is no need to avoid chromium picolinate (FSA 2004).

EFSA (2010). The EFSA evaluated the safety of chromium III as a nutrient added to food for particular nutritional uses and foods intended for the general population (including supplements), with a focus on the potential genotoxicity of chromium III. The EFSA concluded that the safety of chromium III as a nutrient added to food is not of concern, provided that the ingestion of chromium III from the food is not greater than 250 μg per day (which the WHO considered as the level of chromium supplementation that should not be exceeded). The EFSA's conclusion was based on the following: (1) the maximum intake levels for supplemental intake established by the WHO would be in the same order of magnitude as normal dietary intake of chromium in the EU; (2) chromium picolinate (and other sources of chromium III) might cause DNA damage at high concentrations in vitro; (3) the DNA damage that may occur in vitro has not been reported in in vivo genotoxicity assays; (4) chromium III is not carcinogenic; and (5) there is a large margin of safety between an intake of 250 μg per day (4.1 μg per kg per day in a 60-kg adult) and the NOAEL of 6,100 mg chromium picolinate monohydrate per kg per day (727 mg chromium III per kg per day) for mice and of 2,400 mg chromium picolinate monohydrate per kg per day (300 mg chromium III per kg per day) for rats in the long-term studies conducted by the NTP.

CRN Recommendations

The CRN concludes that the available clinical trial data are sufficient to indicate safety for chromium supplements at levels of up to 1,000 µg per day for adults. On the basis of both the large number of clinical trials summarized in Table B-1 from the IOM's 2006 draft monograph and other official reviews of forms of chromium III, CRN sets its UL for chromium supplements at 1,000 µg per day, including the picolinate form and other forms of chromium III.

Quantitative Summary for Chromium

CRN UL, supplemental intake	1,000 µg/day (any chemical form of chromium III)
IOM UL, total intake	Not determined
EC SCF UL, total intake	Not determined
EFSA, maximum added to foods (including food supplements)	250 µg/day
EC supplement maximum	Not determined
EVM, guidance level, total intake	10 mg (10,000 µg)/day

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Copper

Introduction

Copper, like iron and some other elements, is a transition metal and performs at least some of its functions through oxidation-reduction reactions. These reactions involve the transition from Cu^{1+} to Cu^{2+}. There is little or no Cu valence 0 (the metallic form) in biological systems (European Commission, Scientific Committee on Food [EC SCF] 2003).

The essential role of copper was recognized after animals that were fed only a whole-milk diet developed an apparent deficiency that did not respond to iron supplementation and was then recognized as a copper deficiency (Turnlund 1999). The similarity of copper-deficiency anemia and iron-deficiency anemia helped scientists to understand copper's important biological role as the activator of the enzyme ferroxidase I (ceruloplasmin), which is necessary for iron absorption and mobilization from storage in the liver (Linder 1996; Turnlund 1999; EC SCF 2003). Copper activates several enzymes involved in the metabolism of amino acids and their metabolites, energy, and the activated form of oxygen, superoxide. Enzyme activation by copper produces physiologically important effects on connective tissue formation, iron metabolism, central nervous system activity, melanin pigment formation, and protection against oxidative stress.

There are two known inborn errors of copper metabolism. Wilson disease results when an inability to excrete copper causes the element to accumulate, and Menkes disease results when an inability to absorb copper creates a copper deficiency (Turnlund 1994).

Safety Considerations

Copper is relatively nontoxic in most mammals, including humans (Scheinberg and Sternlieb 1976; Linder 1996). Excess copper intakes that cause acute or chronic adverse effects are rare, as absorption is decreased and excretion is increased to maintain homeostasis in response to large amounts of copper. Because of species and dietary differences (e.g., variations in iron, zinc, and molybdenum in the diet), the minimum toxic copper level varies. Additionally, the chemical form of copper has an impact on susceptibility to copper toxicity (EC SCF 2003).

The adverse effects that may occur after acute intake of massive amounts of copper include epigastric pain, nausea, vomiting, and diarrhea (Turnlund 1999; Institute of

Medicine [IOM] 2001). These reactions tend to eliminate the large amounts of ingested copper that caused them and thereby help reduce the risk of its more serious manifestations, which can include coma, liver and kidney pathologies, and death. Adverse effects related to longer-term ingestion of excess copper have been reported for infants in India. These cases of "Indian childhood cirrhosis" arose after milk formula was heated in brass pots, which leached large amounts of copper into the formula (Linder 1996). The intakes of copper associated with these cases are not known. Similar effects can be produced in animals by feeding them diets that contain very large amounts of copper (e.g., 2,000 mg per kg of feed). In humans, chronic copper toxicity has its most pronounced effects on liver function (EC SCF 2003).

Official Reviews

IOM (2001). The IOM reviewed the evidence related to possible adverse effects of copper on the gastrointestinal tract, liver, and other systems. Using data from the clinical trial of Pratt and coworkers, which showed no liver toxicity, the IOM identified a NOAEL of 10 mg per day as supplemented copper gluconate (Pratt et al. 1985). The UF of 1.0, based on a large international database indicating no adverse effects associated with copper intakes of 10 to 12 mg per day, was selected to derive an IOM UL of 10 mg per day. This UL nominally applies to total intakes from all sources, but it was derived from data on supplemental uses of 10 mg per day in persons with unspecified dietary copper intakes. The IOM identified 1.2 to 1.6 mg per day as a typical copper intake from foods. Its report states clearly that the UL does not apply to persons with Wilson disease or any other disorders that cause copper retention and toxicity.

EC SCF (2003). The EC SCF, in preparing its opinion on the tolerable upper intake level of copper, reviewed the evidence related to acute and chronic toxicities caused by excess copper intake. For chronic toxicity, the following possible toxicities were considered: carcinogenicity, genotoxicity, increased risk of coronary heart disease, and neurological disease. The EC SCF also identified a NOAEL of 10 mg per day, based on the same evidence (Pratt et al. 1985) selected by the IOM. Keeping in mind that the body burden of copper increases at different intake levels, the EC SCF selected a UF of 2 to derive a UL of 5 mg per day. It was noted that the 97.5 percentile of copper intake in Europe approaches the UL for adults (i.e., less than 5 mg per day), a matter that was not considered to be of concern.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM reviewed the same human evidence relied upon by the IOM and EC SCF, but elected to derive an SUL from animal studies. Looking at data obtained from a wide range of copper intakes (Herbert et al. 1993), the EVM identified a NOAEL for copper (as copper sulfate) of 16

mg per kg of body weight in male rats. From this NOAEL value, the EVM derived an SUL of 10 mg per day by using a composite UF of 100 and correcting to a 60-kg human body weight. This SUL is intended to apply to total intakes from all sources. The EVM expressed concern that copper intakes from water may reach 6 mg per day in some groups in the United Kingdom.

CRN Recommendations

The NOAEL of 10 mg per day identified by the IOM and the EC SCF was derived from a clinical trial of supplemental copper in subjects with unspecified dietary copper intake. CRN concludes that this value represents the supplemental copper NOAEL from current data. Considering the absence of adverse effects at intakes in the range of 10 to 12 mg per day, and the fact that the usual intake of copper is less than 2 mg, CRN identifies 9 mg as the UL for supplemental copper.

Quantitative Summary for Copper

CRN UL, supplemental intake	9 mg/day
IOM UL, total intake	10 mg/day
EC SCF UL, total intake	5 mg/day
EC supplement maximum	Not determined
EVM SUL, total intake	10 mg/day

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Fluoride

Introduction

Most fluoride in the body is found in bones and teeth, due to its high affinity for calcium and calcium phosphate. Ingestion of and topical treatment with fluoride is effective in inhibiting or partly reversing dental caries. Fluoride deposition into the dental enamel in the form of acid-resistant fluoroapatite crystals, especially during pre-eruptive tooth development, has been a critical factor in reducing the incidence of dental caries during the last few decades. And because it can stimulate growth of new bone, fluoride has been used experimentally to treat osteoporosis (Institute of Medicine [IOM] 1997). Despite these well-documented beneficial effects, there is no scientific agreement that fluoride should be considered an essential element—in other words, necessary for the life of the individual and continuation of the species. Despite these limitations, fluoride clearly is a beneficial nutrient.

Safety Considerations

Fluoride toxicity is well known and has been extensively reviewed (Department of Health and Human Services [DHHS], Public Health Service 1991; IOM 1997; Environmental Protection Agency [EPA] 1987; European Food Safety Authority [EFSA] 2005). The critical adverse effects (i.e., those of significant adverse consequences and occurring at the lowest intakes) are dental fluorosis in children and skeletal fluorosis in adults. Excessive intake in children before their permanent teeth are fully formed can result in dental fluorosis that manifests mainly as mottled brown discoloration and some increase in fragility. Dental fluorosis has been studied in relation to both municipal drinking water fluoridation (for the anticariogenic effect) and naturally occurring high-fluoride water supplies. The maximum fluoride intakes by children that will safely avoid dental fluorosis depend on age and body size, as well as intakes of calcium and other nutrients.

Excessive intake of fluoride by adults results in skeletal fluorosis, which carries an increased risk of bone fracture. The IOM, however, may have somewhat underestimated the potential for fluoride to increase bone fracture risk—8 mg/day. Some epidemiological data suggest that an increased rate of bone fracture is associated with drinking water containing high fluoride concentrations (4 mg per L) and low drinking water calcium concentrations (15 mg per L) (DHHS 1991).

The epidemiological data do not present any clear pattern of association of fluoride intake with cancer risk (DHHS 1991; EFSA 2005). Animal studies are almost all negative for

carcinogenicity of fluoride compounds found in water and food. The sole exception is the finding of "equivocal evidence" of carcinogenicity of sodium fluoride in the male Fisher 344/N rat based on an increased incidence of osteosarcomas (DHHS, National Toxicity Program [NTP] 1990). With the large number of studies performed, a single study that suggests possible significant effects is not surprising. No other data suggest an increased cancer risk related to fluoride consumption.

Official Reviews

IOM (1997). The IOM UL for adults, representing the level at which skeletal fluorosis may be avoided, is 10 mg per day, based on an adult NOAEL of 10 mg and an UF of 1.0. Such a UL was justified by the lack of change in skeletal density found at higher intakes. For younger age groups with incomplete dental enamel development and maturation, the IOM selected dental fluorosis as the critical endpoint. On the basis of dose-response relationship data that indicated lower NOAEL and LOAEL values for these younger age groups, the IOM identified correspondingly lower UL values (0.7 mg per day for infants 0 to 6 months; 0.9 mg for 7 to 12 months; 1.5 mg for 1 to 3 years; and 2.2 mg for 4 to 8 years).

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM considered fluoride for evaluation but declined to review it or offer an opinion because "it is inappropriate to comment on fluoride with regard to food fortification since this [the fluoridation of drinking water] is carried out as a public health measure."

EFSA (2005). The EFSA opinion on fluoride safety was published in 2005, with different UL values for different age groups. For younger children, the critical endpoint for the derivation of the UL was moderate enamel fluorosis, which occurred in less than 5 percent of populations at fluoride intakes of 0.1 mg per kg per day. No UF was applied as the intake was derived from population studies in the susceptible group. Calculated on a body weight basis, the UL was 1.5 mg per day for children ages 1 to 3 years and 2.5 mg per day for children ages 4 to 8 years. For children older than 8 years and adults, the UL was derived from therapeutic studies suggesting an increased risk for skeletal fractures at or above fluoride intakes of 0.6 mg per kg per day. With the application of a UF of 5 to account for the short duration and design of the studies, the UL was determined to be 5 mg per day for children 9 to 14 years and 7 mg per day for individuals 15 years and older.

CRN Recommendations

High intakes of fluoride can have adverse effects on the kidneys and the immune, gastrointestinal, genitourinary and respiratory systems. All of these effects occur at

intakes higher than those that may cause skeletal fluorosis and possibly increase bone fracture risk. Thus, none can be considered the critical effect for identifying an UL. Instead, CRN, in agreement with the IOM and EFSA, identifies skeletal fluorosis as the critical effect in the evaluation of fluoride safety for adults.

The data associated with a daily intake of 1.5 L of fluoridated drinking water suggest that an increased risk of fracture related to skeletal fluorosis might occur with intakes of 6 mg of fluoride or more per day from this source. Thus, if the fluoride intake from foods and nonfluoridated water is approximately 1 mg per day, and the intake from fluoridated toothpaste is approximately 1 mg per day, the addition of these quantities to the 6 mg per day for high-fluoride water suggests that a total intake of 8 mg per day increases the risk of bone fracture in persons whose drinking water has low calcium concentrations. The adult LOAEL, then, is 8 mg per day. This contrasts with the IOM adult NOAEL of 10 mg per day. A UF of 1.3 is adequate for application to a conservative LOAEL, particularly given the IOM's selection of an UF of 1.0 for a NOAEL of 10 mg (producing a calculated UL of 10 mg). Therefore, CRN's calculated UL is 6 mg. CRN does not identify a ULS for adult fluoride supplementation.

Quantitative Summary for Fluoride

CRN UL, total intake	6 mg/day
IOM UL, total intake	10 mg/day
EFSA UL, total intake	7 mg/day
EC supplement maximum	Not determined
EVM SUL and guidance level	Not determined

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Iodine

Introduction

Iodine is an essential element for animals because it is a constituent of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) (Stanbury 1996; Hetzel and Clugston 1999; Institute of Medicine [IOM] 2001; European Commission, Scientific Committee on Food [EC SCF] 2002). Both iodine deficiency and excess have adverse consequences for the thyroid gland. Iodine deficiency not only results in a syndrome known as iodine deficiency disorder (IDD) but can also, in extreme cases, ultimately result in goiter, the overt manifestation of thyroid gland enlargement stimulated by deficiency of the thyroid hormones. This hormone deficiency leads to many adverse effects in addition to gland enlargement, including congenital and developmental defects, poor growth, and mental retardation. Excess iodine—as well as iodine deficiency—can lead to thyroid dysfunction and elevated thyroid stimulating hormone (TSH) levels (Laurenberg et al. 1998).

Iodine occurs in the atmosphere—by evaporation of seawater and industrial sources (EC SCF 2002). Iodine levels in foods and total diets are dependent on geochemical, soil, and cultural conditions. The major natural food sources of iodine are marine fish, shellfish, marine algae, and sea salt. Milk and dairy products contain relatively high amounts derived from iodinated cattle feed supplements and iodine-containing sterilization products.

In mountainous tropical countries, iodine intakes are higher near the coast and lower in high regions where rain has leached much of the iodine from the soil (EC SCF 2002).

Safety Considerations

Except for rare instances of hypersensitivity to iodine, humans are remarkably tolerant of high intakes of iodine (Stanbury 1996; EC SCF 2002). Although toxic effects are not observed in humans until daily intakes have exceeded 10,000 µg, intakes of 2,000 µg should be regarded as excessive and potentially harmful (Hetzel and Clugston 1999). Residents of coastal regions in some areas of Japan have chronic daily intakes of iodine as high as 50,000 to 80,000 µg. Persons who have not been conditioned by iodine deficiency can maintain normal thyroid size and function when they are consuming several milligrams of dietary iodine per day, but previous deficiency can cause hypersensitivity (Hetzel and Clugston 1999). In such situations, hyperthyroidism and iodine-induced thyroiditis may occur when intakes exceed approximately 200 to 300 µg per day. Healthy adults are much less sensitive to excess iodine.

Official Reviews

IOM (2001). The IOM concluded that elevated TSH levels associated with high levels of iodine intake constituted the critical indicator for adverse effects of excess iodine in a healthy adult population. For normal persons who have not been conditioned to iodine deficiency, the IOM identified an LOAEL of 1,700 µg per day. A UL of 1,100 µg of iodine from all sources was derived by applying a UF of 1.5 to the LOAEL. The IOM concluded that the adult iodine intake in the U.S. is usually 240 to 300 µg per day from foods plus another 140 µg from dietary supplements.

EC SCF (2002). The EC SCF utilized iodine intakes of 1,700 and 1,800 µg to establish a UL value, but selected a default UF of 3 to derive a UL of 600 µg per day. The report concluded that dietary intakes are unlikely to exceed 500 µg per day, since the 97.5 percentile intake in European men is 434 µg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM, deciding that neither human nor animal data were sufficient to set a UL value, set a guidance level instead. From several clinical studies of supplemental iodine (Gardner et al. 1988; Paul et al. 1988; Chow et al. 1991), it was concluded that 500 µg of supplemental iodine "would not be expected to have any significant adverse effects in adults." The EVM identified 430 µg as the 97th percentile intake by adults. This led to establishment of guidance levels of 500 µg for supplemental iodine and 930 µg for total intake from all sources. Notably, the EVM did not cite the article by Laurenberg and coworkers (1998) that was relied upon by the IOM and the EC SCF in their calculations.

CRN Recommendations

CRN identifies its NOAEL for iodine as 500 µg per day for supplements and 1,000 µg for total intake. These values are based on the absence of adverse effects in healthy adults given 500 µg of supplement. Although the experimental subjects consumed diets of unknown composition (Gardner et al. 1988; Paul et al. 1988; Chow et al. 1991), their dietary intake of iodine almost certainly did not exceed 500 µg. The NOAEL for supplemental iodine is justified as the CRN UL because adverse effects occur only at 1,700 µg or higher total intake (the LOAEL identified by the IOM and EC SCF) and because dietary intakes almost certainly will not exceed 500 µg.

Quantitative Summary for Iodine

CRN UL, supplemental intake	500 µg/day
IOM UL, total intake	1,100 µg/day
EC SCF UL, total intake	600 µg/day
EC supplement maximum	Not determined
EVM, guidance level	500 µg/day supplemental; 930 µg/day total intake

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Iron

Introduction

Scientists have known since the seventeenth century that iron was present in the blood, but definitive evidence that inorganic iron could be used in the synthesis of hemoglobin was obtained only some seventy years ago (Fairbanks 1999). In addition, iron is an essential component of the myoglobin in muscle, cytochromes, and other enzymes, including the antioxidant enzyme catalase (Yip and Dallman 1996).

Dietary iron occurs in three major forms: ferrous iron (Fe^{2+}), ferric iron (Fe^{3+}), and heme iron (Fe^{2+} chelated into a complex organic compound to complete the heme structure, which occurs in hemoglobin, myoglobin, and certain enzymes). Ferrous iron and ferric iron may be present as part of, or attached to, many different inorganic or organic compounds: the oxide of iron, or complexed with amino acids, citric acid, vitamin C, transferrin, ferritin, and iron-cytochrome reductase ("Iron and iron deficiency" 1998).

Iron deficiency may result from inadequate amounts of dietary iron, depressed or inhibited absorption, or blood loss. Protracted deficiency can lead to a characteristic anemia (microcytic or hypochromic). In more recent studies, iron deficiency has been linked to decreased work performance, altered behavior, decreased intellectual performance, disturbed body temperature regulation, decreased immune function, and decreased resistance to infection (Yip and Dallman 1996; Lynch, 2011).

Bioavailability

The amount of iron in the body is regulated principally by intestinal absorption, transport, storage (mainly in the liver), mobilization, and loss (such as during menstruation) (Yip and Dallman 1996; Fairbanks 1999; Tandara and Salamunic 2012; Collings et al. 2013). In general, the bioavailability of ferrous iron (Fe^{2+}) is somewhat higher than that of ferric iron (Fe^{3+}), and more soluble salts have higher bioavailability than less soluble ones. Heme iron (Fe^{2+}, but bound with the heme molecule unbound from hemoglobin and myoglobin) is more efficiently absorbed than nonheme iron, and heme iron absorption is not limited by the iron absorption control mechanism of the intestine. Depending on the amounts of meats and related products consumed, this extremely efficient absorption of heme iron presents significant problems in identifying the amount of nonheme iron salts that can be considered safe.

In general, ferrous iron, especially the more soluble compounds such as ferrous citrate or ferrous ascorbate, is more easily absorbed than the ferric compounds, which must be reduced from Fe^{3+} to Fe^{2+} before they can be absorbed. Vitamin C is especially effective in enhancing iron absorption not only because it forms soluble complexes with iron but also because it is effective in reducing the ferric form to the ferrous form.

Assuming that ferrous iron is presented to the intestinal mucosa cells, the amount of iron absorbed is regulated by the body's stores of iron—the more stored, the less absorbed. Specifically, the proteins ferritin and transferrin facilitate and regulate iron absorption. Some 4 to 10 percent of dietary nonheme iron is usually absorbed, depending on the specific chemical forms, other dietary components such as vitamin C and amino acids and inhibitors such as phytic acid, and the body stores of iron. Also, low amounts of stomach acid allow the pH of the gastric contents to go up, and Fe^{2+} can be precipitated as the very insoluble compound ferrous hydroxide. This increase in gastric pH also occurs as a result of regular, heavy use of antacid drugs.

The amount of nonheme iron is strongly regulated by the intestinal mucosa (ferritin and then transferrin) to help assure that the total body amount of iron is within an acceptable range. In brief, nonheme iron absorption is strongly regulated.

In contrast, heme iron absorption is not strongly regulated: it is usually on the order of 20 to 25 percent of the heme iron ingested, regardless of the body load of iron. Heme iron is absorbed from meat more efficiently than dietary inorganic iron and in a different manner. Thus, iron deficiency is less frequent in countries where meat constitutes a significant part of the diet. Proteolytic digestion of myoglobin, hemoglobin, and cytochromes results in the release of heme, which is maintained in a soluble form by globin protein degradation products so that it remains available for absorption. Chelators that either diminish or enhance the absorption of inorganic iron have little effect on the absorption of heme iron. Heme enters the small intestinal absorptive cell as an intact metalloporphyrin.

These differences in iron absorption present difficulties that have yet to be significantly addressed by the scientific, policy, and regulatory communities.

Safety Considerations

Almost all supplemental or fortification iron is in the form of one or another ferrous compound. The safety of these amounts depends not only on the body load of iron and the specific chemical form but also on the amount of heme consumed (principally in

animal-based foods such as meats, fish, and poultry). A few heme supplements are available in the U.S. If heme consumption is high, the tolerance for nonheme iron in supplements or fortified foods should be lowered. Few experiments have been conducted to describe and quantify this relationship.

Consequently, policy and regulatory decisions must be made with very incomplete evidence. Because numerous clinical trials have been conducted with various levels of supplemental nonheme iron over short to intermediate periods, some evidence is available that the common dietary variations in heme iron consumption are not great enough to have major effects on the safety of the supplemental nonheme iron. Because of the absences of appropriate quantitative data, it is necessary to assume some average intake of heme that does not vary greatly enough to have public safety impacts—for most people.

For chronic, habitual intake by individuals who do not have any genetic defects that increase iron absorption or retention, iron has shown no adverse effects at levels several times the RDA of 8 mg for men and 18 mg for young women (Institute of Medicine [IOM] 2001). Loss of iron during menstruation accounts for most or all of the difference between the male and female RDAs.

Chronic iron overload has resulted from several conditions or circumstances, including hereditary hemochromatosis, alcoholic liver disease, and excessive intake of dietary iron, especially from home-brewed alcoholic beverages (Fairbanks 1999). Long-term daily ingestions of iron from some home-brewed alcoholic beverages may exceed 100 mg per day. This level of chronic iron intake, at least in combination with chronically high alcohol intake, can lead to Bantu siderosis, a liver disease first discovered in Africa that involves excessive storage of iron and subsequent diseases of the liver and other organs.

Hereditary hemochromatosis, a genetic disorder of iron uptake and storage, has a homozygous frequency of less than 3 to 4 per 1,000 in populations of European extraction (Yip and Dallman 1996). This condition may lead to excessive iron storage even at intake levels recommended for most of the population. There is no clear evidence that carriers for the gene (heterozygous condition) have any increased risk of excessive iron uptake and storage, but such an effect, if any, must be very small compared with the effect in those who are homozygous.

Heart Disease

Select studies on high plasma ferritin levels (Sullivan 1981; Salonen et al. 1992) led some scientists to suggest that dietary iron might be linked to an increased risk of heart disease. This relationship has been contradicted by subsequent evidence and evaluation (Aronow 1993; Baer et al. 1994; Liao et al. 1994; Morrison et al. 1994; Moore et al. 1995; Sempos et al. 1996; Franco et al. 1998; Nasser et al. 1998; Danesh and Appleby 1999; Kaldara-Papatheodorou et al. 2010; Avni et al. 2012) indicating that there is, in fact, no causal relationship. Although some follow-up studies in Europe and Japan support the concept that dietary iron may increase the risk of heart disease (Roest et al. 1999; Tuomainen et al. 1999; Zhang, Iso, et al. 2012), the preponderance of evidence and expert opinion suggests that there is no significant risk (IOM 2001; Expert Group on Vitamins and Minerals [EVM] 2003; European Food Safety Authority [EFSA] 2004).

For prolonged but not chronic use, such as in pregnancy, daily supplements of up to 60 mg are routinely and safely consumed. In other adults, the 95th percentile of supplemental intake is reported as 54 mg for men and 67 mg for women (Stewart et al. 1985). Many high-potency multivitamin and multimineral dietary supplements contain 27 mg of iron. No adverse effects have been attributed to this intake level.

Colonic Cancer

The hypothetical basis on which dietary iron might increase the risk of colonic cancer involves several factors: the catalytic oxidative effects of iron, the procarcinogenic effects of oxidative stress, the association of elevated plasma ferritin values with risk of colonic adenomatous polyps, and the progression of polyps to colonic cancer (Nelson 1992; Tseng et al. 1996). While there is strong evidence for most steps in this mechanistic or associative chain, it does not follow that increases in dietary iron necessarily lead to an increased risk of colonic cancer (Zhang, Giovannucci, et al. 2011).

Dietary iron is absorbed with an efficiency that ranges from as low as 1 or 2 percent (from diets high in inhibitors such as phytic acid) to as high as 30 percent (in pregnant women or those who are iron deficient) (Fairbanks 1999). The mucosal control of iron applies only to nonheme forms, making heme iron absorption usually much more efficient than that of nonheme iron. Regardless of the various absorption factors, however, most ingested iron is not absorbed, giving it the potential to produce oxidative effects in the colonic contents during intestinal transit. The oxidative influences of

unabsorbed iron in the intestine may possibly increase the risk of cancer, but this has not been confirmed.

Acute Iron Poisoning in Children

Acute iron poisoning has occurred in children under three years of age who have accidentally consumed massive amount of iron salts in the form of high-potency (usually 60 or 65 mg), single-nutrient iron supplements (Food and Drug Administration [FDA] 1995), which are usually recommended for prenatal use. The quantities of iron involved in these cases exceed 900 mg in a single ingestion. Such levels of iron override the intestinal regulatory mechanisms and lead to greatly increased plasma levels of iron. No severe adverse effects other than mild gastrointestinal symptoms, however, have been reported in association with acute ingestion of any of the many children's multivitamins that contain iron (most with 27 mg or less iron). The adverse effects that may result from acute ingestion of large amounts of iron have no bearing on the safety of appropriately used iron supplements (Chang and Rangan 2011).

Official Reviews

After a comprehensive review and analysis, the key organizational bodies concerned with vitamin and mineral issues (the IOM, the EVM, and EFSA have found no credible evidence that high iron intake causes any increased risk of cardiovascular disease or cancer in healthy adults (IOM 2001; EVM 2003; EFSA 2004).

IOM (2001). The IOM review identified, based on the clinical evidence by Frykman and coworkers (1994), a significant but low frequency of adverse gastrointestinal effects (constipation and irritation) after administration of iron fumarate, a soluble iron salt, in amounts of 60 mg or more of supplemental iron. Thus, the IOM recommended a supplemental iron LOAEL of 60 mg. To this value, it added the 10 to 11 mg per day dietary iron intake used in the Frykman study (Frykman et al. 1994), setting a total intake LOAEL at 70 mg. Because of the low frequency of the adverse effects and patients' ability to notice and correct for them, the IOM selected a relatively small UF of 1.5 to derive a UL of 45 mg for adults.

EVM (2003). The UK's EVM concluded that the evidence was insufficient to set an SUL value for iron. Instead, it set a guidance level based on some clinical reports of gastrointestinal effects from doses of soluble iron salts containing iron levels as low as 50 mg. The guidance level was calculated by applying a standard default UF of 3 to the low end of the range of doses causing gastrointestinal effect. That is, the guidance level is 50 mg divided by 3, or 17 mg per day. This value is much lower than the IOM value and

contrasts with the EFSA conclusion that the data are simply insufficient to reach any conclusion.

EFSA (2004). EFSA reviewed and evaluated iron safety but concluded that the data were not sufficient to identify a UL value.

CRN Recommendations

A substantial body of evidence supports a NOAEL value for longer-term iron supplementation of 18 to 65 mg per day (with little data on intermediate values) for ferrous and ferric compounds. The data of Frykman and coworkers (1994) indicate a low frequency of mild gastrointestinal effects that are not pathological and are self-limiting due to consumer awareness. This frequency of mild effects represents a nuisance rather than a hazard, and 60 mg of iron qualifies as a supplemental NOAEL if the product label makes the consumer aware of the potential gastrointestinal effects. The large database supporting this conclusion and the complete absence of similar effects at lower supplemental levels, at least when the iron is not taken on an empty stomach, make it reasonable to apply a UF of 1.0. Thus, the CRN ULS for iron is 60 mg per day. It would be appropriate to have a label statement that iron-containing supplements should be taken with food. Note that these recommendations are for ferrous or ferric compounds, not heme iron.

Quantitative Summary for Iron

CRN UL, supplemental intake	60 mg/day
IOM UL, total intake	45 mg/day
EFSA UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level, supplemental intake	17 mg/day

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Manganese

Introduction

Manganese can exist in a number of oxidation states, of which Mn^{2+} is the predominant form in biological systems (European Commission, Scientific Committee on Food [EC SCF] 2000). Manganese is an essential trace element because it is an activator of several metalloenzymes, including arginase, pyruvate carboxylase, glutamine synthetase, and one form of superoxide dismutase (SOD) (Institute of Medicine [IOM] 2001). Manganese also is a nonspecific activator of several other enzymes.

Deficiency of this element has been induced in several animal species by feeding diets low in manganese. Signs of deficiency in animals include impaired growth, skeletal defects, depressed reproductive functions, ataxia in newborns, and defects in metabolism (Keen and Zidenberg-Cherr 1996; Nielsen 1999). The signs and effect of human deficiency have not been clearly established, but some potential cases in adults have shown failure in normal hair pigmentation, dermatitis, and hypocholesterolemia (IOM 2001). Manganese deficiency has been suggested as an underlying factor in the development of joint disease, hip abnormalities, and osteoporosis (Keen and Zidenberg-Cherr 1996).

Because it activates manganese SOD, manganese is necessary for normal antioxidant defenses. However, the practical importance of this effect has not been demonstrated, either because the data on manganese deficiency are inadequate or because other forms of SOD are also active. In animals, manganese protects heart mitochondrial lipids against peroxidation (Malecki and Greger 1996).

Safety Considerations

Manganese is considered to be one of the least toxic of the trace elements when consumed orally (Keen et al. 1994; Keen and Zidenberg-Cherr 1996; Nielsen 1999; EC SCF 2000). This may be attributed to the homeostatic control of manganese absorption that protects the body from exposure to excess manganese (Department of Health and Human Services [DHHS] 2012). In animals, excess manganese has resulted in neurochemical alterations in the brain as well as neuromotor effects and behavioral changes (EC SCF 2000; DHHS 2012). Neurological effects also have been associated with oral exposure to high manganese levels in humans (EC SCF 2000; IOM 2001; DHHS 2012). In contrast to the relatively low toxicity of oral manganese, environmental and workplace manganese exposures (mainly via inhalation) have led to a variety of

severe neurological and brain effects, including ataxia, a pseudo Parkinson's disease, and behavioral changes (Keen et al. 1994). When administered to animals by injection, manganese can produce central nervous system toxicity (Ingersoll et al. 1995).

Epidemiological reports from Greece provide some evidence of adverse neurological effects in high-manganese areas (Environmental Protection Agency [EPA] 1996; Kondakis et al. 1989). The manganese content of well water in the high-manganese area of Greece averaged approximately 2 mg per liter, which translates to an adult lifetime intake of approximately 3 mg per day. Intake of manganese from food in the high-manganese area was initially estimated to be 10 to 15 mg per day, but this was later revised to 5 to 6 mg per day (EPA 1996). These reports suggest that the total intake of manganese in the high-manganese area was either 8 to 9 mg or 13 to 18 mg, depending on which food intake data were used. These discrepancies have led others to conclude that the dietary data were not sufficient to permit reliable estimation of the total oral intake of manganese in these areas (Velazquez and Du 1994; EC SCF 2000).

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EPA (1996). The EPA has set a reference dose for oral manganese equivalent to 10 mg per day for a 70-kg person based on human data NOAEL of 10 mg. Any values that might be selected as LOAEL values are much higher, thus justifying the application of a UF of 1 to the 10 mg per day NOAEL, to derive the maximum of 10 mg per day—an amount calculated to represent a safe oral intake.

EC SCF (2000). The EC SCF reviewed manganese toxicity but declined to set a UL, citing "the limitations of the human data and the non-availability of NOAELs for critical endpoints from animal studies." Based on neurotoxicity findings and the potential increased susceptibility of some population subgroups, the EC SCF noted that "oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit."

IOM (2001). The IOM concluded from the clinical data of Greger (1999) that 11 mg per day of manganese from the consumption of Western-type diets had no adverse effect; they therefore set this amount as the NOAEL. The IOM also identified a LOAEL of 15 mg on the basis of potentially adverse effects upon manganese-dependent SOD**,** as well as other changes (Davis and Greger 1992). With no evidence of toxicity at intakes of less than 11 mg per day, a UF of 1.0 was selected, resulting in an IOM UL for manganese of 11 mg per day.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM concluded that chronic exposure to excess manganese caused neurotoxicity in humans and animals but found the data insufficient to set an SUL. Instead, a guidance level was established based on the data of Vieregge and coworkers, which found no adverse effects from 4 mg of manganese in addition to the manganese present in foods (Vieregge et al. 1995). On the basis of this information, the EVM set guidance levels of 4 mg for supplemental manganese and 12.2 mg for manganese intake from all sources, given an estimated food intake average of 8.2 mg. Because no adverse effects were seen, no correction for uncertainty was deemed necessary. In estimating a mean intake from food of 4.9 mg and from supplements of 10 mg, the EVM noted that the high manganese intake from tea likely has little impact due to limitation of the absorption by the tannins present in tea.

CRN Recommendations

Several types of data show that oral manganese intakes of up to 10 mg per day do not cause adverse effects in adults (World Health Organization 1973; Freeland-Graves et al. 1987; Velazquez and Du 1994; IOM 2001). Epidemiological data related to manganese intakes from well water in Greece do not provide any reliable estimate that contradicts this conclusion. The potential great variability of manganese intake from food and water, as well as factors that may limit manganese absorption, makes it difficult to set a UL for supplemental manganese. The variability in manganese intake from foods would seem to argue for caution on supplemental amounts, but the absence of clinical signs of adverse effects (in contrast to biochemical markers) at intakes of up to 20 mg suggests that such caution is not needed. Considering the low efficiency of manganese absorption and the absence of any credible reports of adverse effects, CRN sets a UL for chronically used supplements at 10 mg per day.

Quantitative Summary for Manganese

CRN UL, supplemental intake	10 mg/day
IOM UL, total intake	11 mg/day
EC SCF UL, total intake	Not determined
EC supplement maximum	Not determined
EVM, guidance level	4 mg/day supplemental; 12.2 mg/day total intake

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Molybdenum

Introduction

Molybdenum is present in food and water in the form of soluble molybdates. It is a component of and/or cofactor for various enzymes in plants and animal organisms (European Commission, Scientific Committee on Food [EC SCF] 2000). In humans, molybdenum acts as a cofactor for several enzymes, including aldehyde oxidase, sulfite oxidase, and xanthine oxidase (EC SCF 2000; Institute of Medicine [IOM] 2001). These and perhaps other functions make it a nutritionally essential element.

Attempts to produce deficiency in experimental animals have succeeded only when the diet contained large amounts of tungsten, an antagonist of molybdenum metabolism (Nielsen 1994, 1996). Molybdenum deficiency in experimental animals inhibits growth and development, especially in prenatal and neonatal stages of development. Human deficiencies of molybdenum function have been linked not to simple dietary deficiency but rather to inborn errors of metabolism, specifically a genetic defect in the molybdenum cofactor that prevents the synthesis of sulfite oxidase, resulting in the accumulation of sulfite, severe neurological damage, and early death (Nielsen 1994, 1999; Johnson, 1997).

Safety Considerations

Most of the toxicity data pertaining to molybdenum in animals is in ruminants, which are susceptible to the adverse effects of molybdenum under conditions of copper deficiency and marginal sulfur amino acid intake (Underwood 1977). However, the basis for toxicity in ruminants is not considered to be of relevance to humans (IOM 2001). In monogastric laboratory animals, variable responses to excess molybdenum have been reported, including growth depression, renal effects, and skeletal abnormalities (EC SCF 2000; IOM 2001). Supplemental molybdenum intakes of 1.6 mg per kg per day and higher from its addition to drinking water adversely impacted several reproductive and developmental parameters in rats (Fungwe et al. 1990). The NOAEL for this study was 0.9 mg molybdenum per kg per day.

Limited data on the toxicity of molybdenum in humans are available. In one study, men who consumed 10 to 15 mg of molybdenum per day for prolonged periods developed abnormally high serum uric acid levels and increased cellular xanthine oxidase activity (Kovalsky et al. 1961). However, serum uric acid levels were not adversely impacted in

another study in which subjects consumed water containing molybdenum at levels providing up to 7 µg per kg per day (Chappell et al. 1979).

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Environmental Protection Agency (EPA 1992). The EPA utilized the epidemiological (human) data of Kovalsky and coworkers (1961) that suggested a LOAEL of 140 µg per kg per day. From this LOAEL value, a reference dose (RfD) was calculated by applying a composite UF of 30 (10 for LOAEL-to-NOAEL extrapolation and 3 for variability within the human population). The resulting value is 5 µg per kg per day, or 350 µg per day for a 70-kg person.

EC SCF (2000). The EC SCF concluded that there were no well-designed human studies that could serve as the basis for a risk assessment of molybdenum. Like the IOM, the EC SCF identified a NOAEL of 0.9 mg per kg per day from rodent reproductive effects (Fungwe et al. 1990) and selected a composite UF of 100 (10 for interspecies differences and 10 for intraspecies variability) to derive a UL of 100 µg per kg per day. To this value, the EC SCF applied a 60-kg body weight to calculate a daily UL of 600 µg for adults.

IOM (2001). The IOM examined the data of Kovalsky and coworkers and found methodological deficiencies extensive enough to preclude use of these data to establish a UL value. Instead of using human data of limited quality, the IOM used animal data as the basis for the UL. The adverse effects of high molybdenum intake on reproduction and fetal development of rats and mice were found to be the most sensitive and therefore served as the basis for the IOM UL. Specifically, the IOM identified a NOAEL of 0.9 mg per kg per day and a LOAEL of 1.6 mg per kg per day for reproductive toxicity in female rats (Fungwe et al. 1990). Using this NOAEL, the IOM selected a composite UF of 30 (10 for interspecies differences and 3 for intraspecies variability) and corrected to a human adult body weight of 68.5 kg to derive a UL of 2,000 µg per day for molybdenum intake from all sources. The IOM estimated the intake of molybdenum from food to be 109 µg for men in the U.S.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM concluded that some human data suggested an increase in gout-like symptoms in populations consuming 1 to 15 mg of molybdenum per day, but that the majority of human data or the relevance of animal data was too uncertain to serve as the basis for an SUL. In the face of such large uncertainties, but with some data suggesting adverse effects at lower levels, the EVM identified a guidance level for total intake that is equal to intake from foods in the

United Kingdom (230 µg per day). The EVM declined to offer guidance about supplemental intakes.

CRN Recommendations

Abnormal plasma uric acid levels were associated with human intakes of 140 µg per kg per day of molybdenum in Kovalsky et al. (1961). However, there has been no corroboration of this finding in other human studies. Although the data are not sufficient for a confident identification of a LOAEL value, CRN prefers to rely upon human rather than animal data when possible. Considering both the large amount of uncertainty and the relatively small intake from foods (109 µg in the U.S.), CRN deems the RfD calculation by EPA to be sufficiently conservative to identify 350 µg as the CRN UL for supplements. This conclusion is more conservative than it would have been had it been based on the animal data used by the IOM and EC SCF for their UL values.

Higher amounts of molybdenum are safe for short periods. For example, 1,490 µg of supplement had no adverse effects in adults over a 24-day treatment period (Turnland et al. 1995).

Quantitative Summary for Molybdenum

CRN UL, supplemental intake	350 µg/day
IOM UL, total intake	2,000 µg/day
EC SCF UL, total intake	600 µg/day
EC supplement maximum	Not determined
EVM, guidance level, total intake	230 µg/day from food; no guidance on supplemental intake

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Selenium

Introduction

Selenium is a trace element that is chemically similar to sulfur and replaces sulfur in the cysteine in certain enzymes (Levander and Burk 1994). Although toxic in large amounts, selenium is a necessary element for humans and some populations suffer from low selenium. The first recognized sign of selenium deficiency, liver necrosis in laboratory animals, was discovered more than 40 years ago. Soon thereafter, combined deficiencies of selenium and vitamin E were shown to cause liver necrosis in rats and swine, exudative diathesis in chickens, and white muscle disease in sheep and cattle. In humans, selenium deficiency is associated with myopathies such as Keshan disease, a cardiomyopathy that is endemic in a few areas of China. This deficiency results from the low selenium content of the soil in certain provinces and thus in the crops that are grown there. Selenium-deficient soils are not limited to China and have been identified in several other countries, including Finland and New Zealand.

The discovery that selenium is a constituent of the antioxidant enzyme glutathione peroxidase provided a biochemical basis that seems to be at least partly responsible for the essentiality of selenium (Centers for Disease Control [CDC] 2012). More recently, its role in thyroid gland activity was demonstrated, specifically as part of the active center of the enzyme type I iodothyronine deiodinase, which converts the prohormone thyroxine (T4) to the active hormone triiodothyronine (T3).

Dietary forms of selenium that are nutritionally useful include the inorganic forms selenite and selenate and the organic forms selenomethionine and selenocysteine. Selenium can be incorporated into growing yeast, which then provides nutritionally useful forms of selenium for animals and humans. Much of the selenium in yeast is selenomethionine, a form that is virtually 100 percent absorbed after oral ingestion; selenomethionine is readily converted to selenocysteine—the form incorporated into certain enzymes. Average total dietary selenium intakes in the U.S. have been estimated at 100 and 70 μg per day for men and women, respectively (CDC 2012).

The epidemiological association of higher selenium intakes with reduced cancer risk and the antioxidant role of selenium in glutathione peroxidase (as well as several other possible mechanisms) have provided a basis for research on possible anticarcinogenic effects of selenium. Several selenium compounds have been shown to have

antitumorigenic activities in a variety of animal models when administered at levels greater than those associated with nutritional need (Rayman 2012).

Two clinical intervention trials published in the mid-1990s were designed to determine whether selenium in combination with other nutrients would reduce cancer risk. In one, 50 µg selenium (in yeast), in combination with vitamin E and beta-carotene, moderately reduced the risk of total mortality, total cancer mortality, and stomach cancer mortality (Blot et al. 1993). In the other, inorganic selenium—together with a wide spectrum of other minerals and vitamins—did not significantly protect against cancer (Li et al. 1993). An additional, placebo-controlled, randomized clinical trial was stopped for ethical reasons after it became clear that treatment with 200 µg of selenium in yeast had significantly decreased lung cancer and overall cancer mortality, as well as the incidence of colorectal and prostate cancer (Clark et al. 1996a, 1996b; Combs and Clark 1997). The amount of selenium used in the study (200 µg) was nearly 3 times the adult male RDA (70 µg) (Institute of Medicine [IOM] 2000). The primary objective of the trial was to determine the effect of selenium on nonmelanoma skin cancer, but there was no effect, either negative or positive, on that disease.

Safety Considerations

Excess selenium intake from consumption of seleniferous plants by animals produces a wide range of adverse effects (National Research Council 1983). Chronic toxicity signs in livestock include cirrhosis, lameness, hoof malformations, hair loss, and emaciation. In laboratory animals, the signs most commonly include cirrhosis. The dietary level of selenium recognized to produce adverse effects in farm animals is 4 to 5 µg or higher per gram dry weight of diet.

One episode of human poisoning by selenium involved a manufacturing error that resulted in a dietary supplement product containing approximately 200 times the amount of selenium declared on the label (MacFarquhar et al. 2010; Aldosary et al. 2012). Adverse effects occurred within a few weeks and included changes in the hair, nails, and liver. Human selenium poisoning in a high-selenium area of China also produced adverse effects on the nails, skin, nervous system, and teeth (Yang et al. 1983). These occurred in susceptible persons with intakes of 910 µg per day or more. No such results have been associated with lower levels of intake, but the ratio of plasma selenium to erythrocyte selenium has been found to increase with dietary intakes of 750 µg per day or more (Yang, Yin, et al. 1989). Human surveys in seleniferous areas of the U.S. have failed to find any signs of selenium intoxication with intakes up to slightly more than 700 µg per day (Longnecker et al. 1991). Because not all the chemical forms of selenium in foods grown in seleniferous areas are known, the human data on adverse effects from

chronically high intakes apply only to total dietary selenium and not to any specific form. No adverse effects were observed in the 8- to 10-year clinical trial by Clark and coworkers (Clark et al. 1996a, 1996b; Combs and Clark 1997) at daily supplemental intakes of 200 µg selenium in yeast. Most of the selenium in this yeast preparation was in the form of selenomethionine.

Ultimately, the adverse effects established in a few individuals at chronic dietary intakes of 910 µg per day qualify that value for identification as the selenium LOAEL. The data of Yang, Yin, et al. (1989) did not find any overt adverse effects, but did find an increase in the ratio of plasma selenium to erythrocyte selenium at intakes of 750 µg per day. Although this change in ratio is not in itself an adverse effect, it may indicate that the ability to eliminate excess selenium is nearly saturated. Application of regression methods to the data of Yang, Zhou, et al. (1989) and Yang, Yin, et al. (1989) supports a NOAEL for total dietary selenium of 853 µg per day in the Chinese adult of 55 kg weight (Combs 1994; Poirier 1994).

Cancer

A number of observational studies suggested that death rates from cancer, including lung, colorectal, and prostate cancer, are lower among people with higher blood levels or intakes of selenium (Patterson and Levander 1997; Russo et al. 1997; Young and Lee 1999). Some clinical trials have indicated that increased selenium intakes may lead to lower risks of a number of types of cancer (Clark et al. 1996a, 1996b), and epidemiological data (actually a nested case control study as a later analysis of data from a large clinical trial—the Health Professionals Follow Up Study) suggested that selenium was associated with a lower incidence of type II diabetes (reviewed and summarized by Rajpathak et al. 2005). To more thoroughly test these possible relationships, two large clinical trials—the SELECT trial (Lippman et al. 2009) and the SU.VI.MAX study (Hechberg et al. 2004)—were conducted. Both trials monitored type II diabetes as well as a number of types of cancer.

The design of the SELECT trial was driven primarily by the results of lower rates of several cancers but not the one most expected—skin cancer—in the selenium trial of Clark et al. (1996a, 1996b) and by the vitamin E results (lower rates of prostate cancer) in the ATBC study (Heinonen et al. 1998). Although the Clark study had provided 200 µg selenium as selenized yeast (yeast grown in a high-selenium medium), the SELECT trial provided selenium (in the same amount) as selenomethionine. The 200 µg dose is well above the IOM's RDA (55 µg) and well below its UL (400 µg).

The ATBC study found a 32 percent decrease in clinically evident prostate cancer among those taking alpha-tocopherol (50 IU), compared with the placebo group; mortality from prostate cancer was 41 percent lower in those taking vitamin E. The SELECT trial did not find any protection against prostate cancer by either selenium or vitamin E (Lippman et al. 2009).

The SU.VI.MAX study examined the effects of a complex supplement containing moderate amounts of vitamin E and C, beta-carotene, zinc, and selenium versus placebo on the risk of chronic diseases such as cancer, cardiovascular disease, and diabetes. Men who began the study with normal (<3 ng per ml) prostate specific antigen (PSA) levels had their prostate cancer risk reduced by half (Meyer et al. 2005). In men whose PSA levels were elevated (>3 ng per ml) at the beginning of the study, however, use of the supplement was associated with a nonsignificant small increase in prostate cancer risk. Overall, the SU.VI.MAX data indicate that the supplement was highly protective for men with low PSA levels and that it either had no impact or possibly caused a small increase in risk for those with high PSA levels.

More recently, the SELECT trial data (Lippman et al. 2009) indicate no significant overall effect on prostate cancer risk for selenium given to men, many of whom may have had elevated PSA levels (African American men ages 50 and older and white men ages 55 and older).

In summary, the SELECT and SU.VI.MAX results together indicate that a selenium-containing supplement is protective for men with low PSA levels, but is either non-effective or may carry a very small risk for those with higher PSA levels. In addition, data on selenium concentrations in toenail clippings suggest that increased selenium intake over a narrow but relatively low intake range is highly protective against prostate cancer risk (Hurst et al. 2012).

Type II Diabetes

Evidence is mixed and seemingly contradictory on the effect of selenium intake on type II diabetes. Rajpathak et al. (2005) reported that levels of toenail selenium are lower among diabetic men with or without cardiovascular disease than among healthy controls. The odds ratio (similar to relative risk) was 0.45 for the highest compared with the lowest quartile of toenail selenium concentration.

More recently, Bleys et al. (2007a) examined data from the Third National Health and Nutrition Examination Survey (a large, multiyear observational, or epidemiological,

study) and found that adults with diabetes had very slightly higher serum selenium concentrations compared with nondiabetics. Before the data were adjusted for a number of factors (gender, age, ethnicity, etc.), the differences were very small and nonsignificant. After adjustment, the mean serum selenium concentrations of selenium were as follows: diabetics 126.8 ng per ml, and nondiabetics 124.7 ng per ml. Because of the large number of persons evaluated, this small difference was labeled as "significant" ($P = 0.02$). A follow-up publication (Bleys et al. 2007b) declared in the article title that these data are "more bad news for supplements."

Several factors fail to support or even contradict the validity of the Bleys et al. observations and conclusions. First is the lack of biological plausibility; it is unlikely that such small differences in selenium concentrations would have a causal relationship to diabetes. Second, there is no evidence of a dose-response relationship. Only one clinical trial (Stranges et al. 2007) seems to support Bleys and coworkers. Moreover, newer observational studies continue to support a substantial effect of higher selenium intakes as being protective against type II diabetes (Park et al. 2012).

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IOM (2000). The IOM judged the reexamination of selenium intakes by Yang and Zhou (1994) to identify a NOAEL for selenium of 800 μg per day. A UF of 2 was selected to provide protection for sensitive individuals, resulting in a UL of 400 μg selenium per day for adults for total oral intake from all sources. The IOM has not expressed an opinion on safe levels for selenium supplementation, except that implied by a total intake UL of 400 μg.

European Commission's Scientific Committee on Food (EC SCF 2000). The EC SCF considered the data of Yang, Yin, and coworkers (1989) sufficient to identify a NOAEL of 850 μg per day. A UL of 300 μg was derived from this NOAEL by application of a UF of 3. The EC SCF noted that this 300 μg UL was supported by the absence of adverse effects in a clinical trial by Clark and coworkers involving a supplement of 200 μg and diets of approximately 100 μg (1996a, 1996b). The EC has not set a regulatory maximum for selenium added to supplements as of the writing of this book.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM did not identify a NOAEL but considered the studies of Yang and coworkers (Yang, Yin, et al. 1989; Yang, Zhou, et al. 1989) to support a LOAEL of 910 μg per day. Furthermore, the EVM, applying a UF of 2 to a LOAEL for a large population with a lifetime of exposure, derived an SUL for selenium of 450 μg.

The EVM noted that its SUL is consistent with the clinical trial data by Clark and coworkers. These expert groups have not offered any explanations for why their NOAEL, LOAEL, and uncertainty values are so different.

CRN Recommendations

The exact forms of selenium consumed by the Chinese population in the epidemiological studies are not known, but it seems likely that much of it would have consisted of selenomethionine, as in the clinical trial by Clark and coworkers. Considering the variability of dietary intakes, a supplemental selenium NOAEL of 200 μg is identified from the clinical trial data. Based on the absence of adverse effects at this supplemental level, and on the substantial margin of safety it provides below levels associated with adverse effects, a UF of 1.0 is sufficient, and the CRN UL for selenium supplements is determined to be 200 μg per day.

When dietary selenium is 100 μg per day, the CRN ULS identified by this direct method for selenium supplementation safety would result in a total intake of 300 μg—equivalent to the SCF UL. Somewhat larger amounts should be safe but the margin of safety would be less generous.

Quantitative Summary for Selenium

CRN UL, supplemental intake	200 μg/day
IOM UL, total intake	400 μg/day
EC SCF UL, total intake	300 μg/day
EC supplement maximum	Not determined
EVM SUL	350 μg/day supplemental intake; 450 μg/day total intake

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Zinc

Introduction

Zinc is an essential element that demonstrates a classic U-shaped dose-response curve in which adverse effects are associated with receiving either too little or too much zinc. Zinc deficiency resulting from inadequate dietary intake can lead to a variety of physiological and developmental impairments, as evidenced by areas of endemic hypogonadism and dwarfism in rural Iran (Cousins 1996; King and Keen 1999). Conditioned (secondary) zinc deficiency related to diseases, iatrogenic causes, impaired absorption, or excess zinc loss can also result in a variety of negative health effects.

Zinc is essential for the functions of numerous enzymes, including many involved in acid-base balance, amino acid metabolism, protein synthesis, and nucleic acid synthesis and function. For example, a zinc-dependent enzyme facilitates the conversion of food forms of folic acid (pteroylpolyglutamates) to free folic acid (pteroylmonoglutamate) to permit the body's utilization of food folates. Subsequently, the conversion of pteroylheptaglutamate to free folic acid is impaired with zinc deficiency. Experimental zinc deficiency has also been correlated with reproductive failure, loss of epidermal integrity, and immune dysfunction.

Safety Considerations

Zinc toxicity can occur either acutely or chronically. Acute zinc toxicity includes nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headache (Institute of Medicine [IOM] 2001). The acute effects of zinc excess typically result from ingesting gram quantities of zinc, which could occur by consuming 40 to 60 servings of a typical multivitamin that provides RDA levels of essential nutrients.

Chronic adverse effects of zinc excess are more subtle. The IOM set its UL value based on a clinical trial in which 60 mg of zinc produced an inhibition of copper-dependent superoxide dismutase. However, the researchers did not determine how much reserve functional capacity is available for this enzyme and whether a small decrease in activity would have any relevant clinical impact. The IOM applied a UF of 1.5 to calculate an adult UL of 40 mg zinc per day.

Supplemental zinc has been shown to influence several biomarkers that may have clinical relevance in certain populations. Zinc supplements of 150 mg per day for 6 weeks have been shown to suppress lymphocyte stimulation response, thereby compromising immune

function in healthy subjects (Chandra 1984; Greger 1994). Zinc supplements of 50 mg or more per day have been shown to decrease serum HDL cholesterol levels (Hooper et al. 1980; Freeland-Graves et al. 1982; Black et al. 1988). Total intakes of 60 mg of zinc decreased levels of copper (Fischer et al. 1984) and iron (Yadrick et al. 1989).

There are several medications that can interact with zinc, including antibiotics such as quinoline compounds and penicillamine, as well as several diuretics, such as hydrochlorothiazide. Zinc supplementation can interfere with the activity of medications, or in some cases medication can result in zinc depletion. A full discussion of drug-nutrient interactions is beyond the scope of this report, and individuals taking prescription medication should be advised to consult with their health care provider about potential drug-nutrient interactions.

Certain zinc–folic acid interactions are well documented (Butterworth and Tamura 1989). But the crucial issue is whether higher intakes of either zinc or folic acid may disrupt the bioavailability or function of the other and, if so, what the intakes associated with such effects are. Some reports of zinc–folic acid interactions suggest the possibility that supplemental folic acid could adversely affect zinc nutriture (Milne et al. 1984; Mukherjee et al. 1984; Simmer et al. 1987), but more recent reports have not uncovered any such interaction (Tamura et al. 1992; Kauwell et al. 1995). There are no Medline reports of high zinc intakes causing adverse effects through an antagonism of folic acid. Reports of anemia related to zinc intakes above 110 mg per day all describe the microcytic, hypochromic anemia associated with copper deficiency, a condition that could also interfere with iron utilization (Frambach and Bendel 1991; Gyorffy and Chan 1992; Summerfield et al. 1992; Greger 1994).

Certain chemical similarities cause zinc and copper to interact extensively (King and Keen 1999). Large quantities of zinc can interfere with copper uptake and modify copper binding, and this effect has been used in treating Wilson disease, a defect that leads to excessive copper storage. Iron can interfere with zinc absorption when zinc is administered as a solution, but such interference has not manifested itself when zinc is consumed as part of a meal. Although high levels of calcium can also interfere with zinc absorption, the effect has no demonstrated practical importance.

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IOM (2001). The IOM found the adverse effects of excess zinc to include a suppressed immune response, decreased HDL cholesterol levels, and a reduced copper status. The IOM did not find adverse effects on human reproduction from excess zinc in their study.

Of the various effects, the IOM selected the reduced copper status as the critical effect for deriving a UL for zinc. Specifically, the IOM used the data showing suppression of copper-dependent superoxide dismutase at 50 mg of zinc supplementation (Yadrick et al. 1989) to identify a LOAEL. Although no zinc intake from food was identified by Yadrick and coworkers, the IOM used population data to estimate a dietary zinc intake of 10 mg for the study. Thus, the IOM identified a LOAEL of 60 mg per day for total intake from all sources. A UF of 1.5 was selected to correct for uncertainty in extrapolation from a LOAEL to a NOAEL; the UF of 1.5 was judged to be adequate because reduced copper status is rare. Thus, the IOM UL for zinc is 40 mg per day for total intake from all sources.

European Commission, Scientific Committee on Food (EC SCF 2003). The EC SCF identified a NOAEL for zinc of approximately 50 mg per day. This NOAEL represents an overall conclusion based upon several studies. Although zinc intakes as low as 18.2 mg may decrease copper retention (Festa et al. 1985), this effect is readily corrected by adequate copper intake. Studies looking at the interplay between zinc and copper (Davis et al. 2000; Milne et al. 2001) indicate that copper balance and other indicators of copper status can be maintained when zinc intake is as high as 53 mg. No adverse effects were observed with 30 mg of supplemental zinc when dietary zinc was near 10 mg (Bonham et al. 2003a, 2003b). From these data collectively, the EC SCF identified its NOAEL of 50 mg of zinc and proposed a UF of 2 to derive a UL of 25 mg for total intake from all sources.

Expert Group on Vitamins and Minerals (EVM 2003). The UK's EVM selected a LOAEL of 50 mg for supplemental zinc based on several studies (Black et al. 1988; Yadrick et al. 1989; Cunningham et al. 1994; Davis et al. 2000). To extrapolate from a LOAEL to a NOAEL, the EVM selected a UF of 2, resulting in a derived SUL of 25 mg per day for supplemental zinc. The total daily intake of 42 mg per day would not be expected to result in any adverse effects

CRN Recommendations

There are no known adverse effects of zinc at chronic supplemental levels of 30 mg per day (Bonham et al. 2003a, 2003b), and this level provides a substantial margin of safety below the levels associated with adverse effects (at least 50 mg of supplemental zinc). Therefore, 30 mg per day is identified as the CRN UL for supplements. Assuming a dietary zinc intake of 10 mg, the CRN UL for supplements is exactly compatible with the 40 mg IOM UL for total intake. The CRN value is only slightly higher than the 25 mg supplemental SUL set by the EVM.

Quantitative Summary for Zinc

CRN UL, supplemental intake	30 mg/day
IOM UL, total intake	40 mg/day
EC SCF UL, total intake	25 mg/day
EC supplement maximum	Not determined
EVM, guidance level	25 mg/day supplemental intake; 42 mg/day total intake

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Acronyms, Abbreviations, Measurements and International Comparison Charts

List of Acronyms and Abbreviations

ADI	acceptable daily intake
AI	acceptable intake
alpha-TE	alpha-tocopherol equivalent
ANS	EFSA Panel on Food Additives and Nutrient Sources Added to Food
AREDS	Age-Related Eye Disease Study
ASEAN	Association of South East Asian Nations
ATBC	Alpha-Tocopherol Beta-Carotene Cancer Prevention Study
BMD	benchmark dose
CARET Study	Carotenoid and Retinol Efficacy Trial
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CDC	Centers for Disease Control
CHAOS	Cambridge Heart Antioxidant Study
DFE	dietary folate equivalent
DRI	dietary reference intake
EC SCF	European Commission's Scientific Committee on Food
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
EVM	Expert Group on Vitamins and Minerals
FAO	Food and Agriculture Organization of the UN
FASEB	Federation of American Societies for Experimental Biology
FDA	U.S. Food and Drug Administration
FNB	Food and Nutrition Board of the Institute of Medicine
FSA	UK Food Standards Agency
GL	guidance level
HHS	U.S. Department of Health and Human Services
HOI	highest observed intake
HOPE	Heart Outcomes Prevention Evaluation Study
IOM	Institute of Medicine
IRIS	Integrated Risk Information System
IU	international unit
IVACG	International Vitamin A Consultative Group

List of Acronyms and Abbreviations

LOAEL	lowest-observed adverse-effect level
LSRO	Life Sciences Research Office
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NIH	National Institutes of Health
NOAEL	no-observed adverse-effect level
NTP	National Toxicology Program
OSL	observed safe level
PHS	Physicians' Health Study
PRI	population reference intake
PSA	prostate specific antigen
RAE	retinol activity equivalent
RDA	recommended daily allowance
REACT	Roche European American Cataract Trial
RfD	reference dose
RNI	recommended nutrient intake (UK)
SCOGS	Select Committee on GRAS Substances
SOD	superoxide dismutase
SUL	safe upper level
UF	uncertainty factor
UL	upper level or tolerable upper intake level
ULS	upper level for supplements
WAVE	Women's Angiographic Vitamin and Estrogen Trial
WHI	Women's Health Initiative
WHO	World Health Organization

Units of Measure

µg	microgram
g	gram
kg	kilogram
mg	milligram
mL	milliliter

International Comparisons:

CRN ULS, US IOM UL, EC SCF/EFSA UL, UK EVM SUL or GL for Adults

Vitamins

Nutrient	CRN ULS[1] (amount/day)	US IOM UL[2] (amount/day)	EC SCF/EFSA[3] UL (amount/day)	UK EVM SUL[4] or GL[5] (amount/day)
Vitamin A (retinol and its esters)	3,000 µg (10,000 IU) w/low dietary retinol; 1,500 µg (5,000 IU) w/high dietary retinol	3,000 µg	3,000 µg	1,500 µg total (GL) (for bone effects)
Beta-carotene	25 mg non-smokers; smokers should not use	Not determined	Not determined	7 mg supplement (SUL); smokers should not use
Vitamin D	250 µg (10,000 IU)	100 µg (4,000 IU)	100 µg (4,000 IU)	25 µg (1000 IU) supplement (GL)
Vitamin E	1,000 mg (1,600 IU)	1,000 mg	300 mg	540 mg supplement (800 IU) (SUL)
Vitamin K	10 mg	Not determined	Not determined	1 mg supplement (GL)
Vitamin C	2,000 mg	2,000 mg	Not determined	1,000 mg supplement (GL)
Vitamin B1 (Thiamin)	100 mg	Not determined	Not determined	100 mg supplement (GL)
Vitamin B2 (Riboflavin)	200 mg	Not determined	Not determined	40 mg supplement; 43 mg total (GL)
Nicotinic acid	500 mg[6]; 250 mg SR[7]	35 mg[8,9]	10 mg[9]	17 mg[9] supplement (GL)
Nicotinamide	1,500 mg	35 mg[8]	900 mg	500 mg supplement; 560 mg total (GL)
Vitamin B6 (Pyridoxine)	100 mg	100 mg	25 mg	10 mg supplement (SUL)
Folic acid	1,000 µg	1,000 µg	1,000 µg	1,000 µg supplement (GL)
Vitamin B12	3,000 µg	Not determined	Not determined	2,000 µg supplement (GL)
Biotin	2,500 µg	Not determined	Not determined	900 µg supplement (GL)
Pantothenic Acid	1,000 mg	Not determined	Not determined	200 mg supplement; 210 mg total (GL)

1 ULS = CRN's Upper Level for Supplements

2 UL = Tolerable Upper Intake Level (applies to total intake unless specified otherwise)

3 EFSA (European Food Safety Authority) assumed this assessment function in place of EC SCF in January 2004

4 SUL = Safe Upper Limit (may apply to either total or supplemental intake, as specified)

5 GL = Guidance Level (may apply to either total or supplemental intake, as specified)

6 Based on liver and gastrointestinal toxicity

7 SR = slow-release (time-release) formulations of nicotinic acid

8 IOM UL for niacin is set for both nicotinic acid and nicotinamide

9 Based on vasodilative flushing reaction

International Comparisons:

CRN ULS, US IOM UL, EC SCF/EFSA UL, UK EVM SUL or GL for Adults

Minerals and Trace Elements

Nutrient	CRN ULS[1] (amount/day)	US IOM UL[2] (amount/day)	EC SCF/EFSA[3] UL (amount/day)	UK EVM SUL[4] or GL[5] (amount/day)
Calcium	1,500 mg	2,500 mg (19-50 yrs)	2,500 mg	1,500 mg supplement (GL)
Phosphorus	1,500 mg	4,000 mg	Not determined	250 mg supplement; 2,400 mg total (GL)
Magnesium	400 mg	350 mg nonfood sources	250 mg nonfood sources	400 mg supplement (GL)
Potassium	1,500 mg (500 mg, 3x per day)	Not determined	Not determined	3,700 mg supplement (GL)
Boron	6 mg	20 mg	10 mg	9.6 mg total (SUL)
Chromium	1,000 µg (any form of Cr III)	Not determined	Not determined	10 mg (10,000 µg) total (GL)
Copper	9 mg	10 mg	5 mg	10 mg total (SUL)
Fluoride	No ULS (UL= 6 mg)	10 mg	7 mg (>15 yrs)	Not determined
Iodine	500 µg	1,100 µg	600 µg	500 µg supplement; 930 µg total (GL)
Iron	60 mg (full stomach)	45 mg (empty stomach)	Not determined	17 mg supplement (GL)
Manganese	10 mg	11 mg	Not determined	4 mg supplement; 12.2 mg total (GL)
Molybdenum	350 µg	2,000 µg	600 µg	230 µg food (GL)
Selenium	200 µg	400 µg	300 µg	350 µg supplement; 450 µg total (SUL)
Zinc	30 mg	40 mg	25 mg	25 mg supplement; 42 mg total (SUL)

[1] ULS = CRN's Upper Level for Supplements

[2] UL = Tolerable Upper Intake Level (applies to total intake unless specified otherwise)

[3] EFSA (European Food Safety Authority) assumed this assessment function in place of EC SCF in January 2004

[4] SUL = Safe Upper Limit (may apply to either total or supplemental intake, as specified)

[5] GL = Guidance Level (may apply to either total or supplemental intake, as specified)

About the Council for Responsible Nutrition

The Council for Responsible Nutrition (CRN), founded in 1973, is based in Washington, D.C., and is the leading trade association representing dietary supplement manufacturers and ingredient suppliers. CRN member companies produce a large portion of the dietary supplements marketed in the United States and globally. The companies manufacture popular national brands as well as the store brands marketed by major supermarkets, drug stores and discount chains. They also market products through natural food stores and mainstream direct selling companies. In addition to complying with a host of federal and state regulations governing dietary supplements, the manufacturer and supplier members also agree to adhere to voluntary guidelines for manufacturing and marketing and agree to comply with CRN's Code of Ethics.

CRN's mission is to sustain and enhance a climate for its member companies to responsibly develop, manufacture and market dietary supplements and nutritional ingredients. CRN provides its member companies with expertise and action in the areas of scientific and regulatory affairs, government affairs, media outreach and communications, and international affairs. CRN takes a leadership role to advocate for public policy based on sound science that permits consumers to have access to a wide variety of high quality, safe and beneficial dietary supplements.

CRN's staff includes recognized scientific and regulatory experts, including those who participated in revising this handbook:

John Hathcock, Ph.D., the author of *Vitamin and Mineral Safety*, currently works as a consultant for CRN, having formerly served as its senior vice president, Scientific and International Affairs. Dr. Hathcock has decades of experience in evaluating the safety of nutrients and other dietary ingredients, having been a professor at Iowa State University and a senior scientist at the Food and Drug Administration before joining CRN in 1995.

Douglas "Duffy" MacKay, N.D., senior vice president, Scientific & Regulatory Affairs, is a licensed naturopathic doctor who has served as a medical consultant to companies in the dietary supplement industry and who also has hands-on experience as a practitioner of integrative medicine. He oversees CRN's scientific and regulatory department, and served as editor of the third edition of *Vitamin and Mineral Safety*.

James C. Griffiths, Ph.D., vice president, Scientific & International Affairs, has more than 25 years of experience in the area of food safety and regulatory affairs. Prior to joining CRN, he spent five years with the United States Pharmacopoeia (USP) where he managed food and dietary supplement strategic initiatives, including those related to global food and dietary supplement alliances. He began his career as a regulatory review toxicologist at the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition. In addition to reviewing and updating the scientific literature for this new handbook, he authored the foreword.

Andrea Wong, Ph.D., vice president, Scientific & Regulatory Affairs, has regulatory expertise that includes a working knowledge of international health claims and preparation of technical submissions to international regulatory agencies. She helped review and update the scientific literature for this book.

Haiuyen Nguyen, associate director, Scientific & Regulatory Affairs, provides research assistance to the scientists at CRN and oversees department projects. For this book, she oversaw production schedules and was involved in updating the research.

Additional information about CRN is available on the website: www.crnusa.org.

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