Approaches to Risk Assessment for Macronutrients and Amino Acids

Joseph V. Rodricks

ENVIRON International Corporation, Arlington, VA 22203

ABSTRACT Risk assessment is a well-established framework for organizing and evaluating diverse, and sometimes conflicting information, to assess the likelihood that agents in the environment may harm human health under known or expected conditions of exposure. Risk assessments are used by regulatory and public health officials to guide judgments and actions regarding the need for risk reduction and the appropriate means to achieve it. These judgments and actions are called risk management, and are guided by law, historical precedent and public health, economic and social concerns. Those in the nutrition community who have been called upon to make recommendations regarding adequate nutrient intakes have long been engaged in the practice of risk assessment. That is, they have assessed the harmful health effects of inadequate intakes and defined intakes likely to avoid such harm. During the past decade attention has turned to the potential health risks of excessive nutrient and nutritional supplement intakes. A recent study released by a committee of the Institute of Medicine illustrates the difficulties in deriving risk-based upper levels of intake for nutrients and nutritional supplements. Amino acids were the subject of extensive discussions by this committee, but in no case was an upper level of intake recommended. It is clear that the extent of scientific investigation of the harmful effects of amino acids has been highly uneven, and that significant questions remain regarding the appropriate methodologies to study such effects.

KEY WORDS: • toxicity • hazard • risk • risk assessment • macronutrients • amino acids • upper levels

Risk assessment is a framework used to organize and evaluate diverse, complex and sometimes conflicting information bearing on the potential for agents in the environment (chemical, biological, physical) to cause harm to human health. The risk assessment process focuses on the range of human exposures to particular agents, and seeks to ascertain whether any significant part of that range is likely to be associated with an increased probability of harm. If such a potentially harmful range of exposures is revealed, strategies for reducing risk to acceptable levels are considered. The process of deciding how and to what extent risks are to be mitigated is in the realm of risk management, and is typically undertaken by regulatory and other officials charged with enforcing public health protection laws. Risk management policies will be sensible and beneficial only to the extent that they are based on scientifically reliable risk assessments.

With respect to chemical substances, risk assessment, as it is now conceived, has been a major force in regulation for about 25 years. It has been principally directed at health hazards caused by the toxic and carcinogenic properties of what shall be referred to as “foreign” compounds, i.e., substances that are not normal constituents of the body or that do not play any necessary physiological role. “Foreign” chemicals, or xenobiotics, include most food additives, pesticides and food contaminants, pharmaceuticals, contaminants of air, water and soils, and most occupational and consumer product exposures. All of these categories of substances are under the risk assessor’s microscope, and decision making regarding permitted human exposures to them is heavily risk based, almost completely so in the U.S. and increasingly so in Europe and elsewhere (1,2).

Those in the nutrition community who have been called upon to make recommendations regarding adequate nutrient intakes have also been engaged in risk assessment; that is, they have for many years been using information from many types of investigations to assess the likelihood of harmful health effects from inadequate nutrient intakes, and to identify levels of intakes necessary to avoid such harm. Risk management officials use this information for dietary planning, public education and food labeling. In more recent years public health attention has turned to the possibility that excessive nutrient intake may also increase health risks, and the steep rise in use of nutritional and dietary supplements has heightened that attention (3).

The increasing need to consider the possibility of increased health risks from intakes of nutrients and nutritional supplements in excess of adequate levels, and from non-nutritional dietary supplements of many types, prompted a search for an appropriate assessment methodology. In the U.S., this search was taken up in 1998 by a committee of the

1 Presented at the conference “The Second Workshop on the Assessment of Adequate Intake of Dietary Amino Acids” held October 31–November 1, 2002, in Honolulu, Hawaii. The conference was sponsored by the International Council on Amino Acid Science. The Workshop Organizing Committee included Vernon R. Young, Yuzo Hayashi, Luc Cynober and Motoni Kadowaki. Conference proceedings were published in a supplement to The Journal of Nutrition. Guest editors for the supplement publication were Dennis M. Bier, Luc Cynober, Yuzo Hayashi and Motoni Kadowaki.

2 To whom correspondence should be addressed. E-mail: jrodricks@environcorp.com.
Institute of Medicine (IOM) on dietary reference intakes (DRI), under the leadership of Vernon Young. The sub-committee responsible for examining the questions of excess nutrient intakes (IOM Subcommittee on Upper Levels) adopted the very same risk assessment framework long in use for foreign chemicals (3,4). This article describes the issues and difficult challenges that arise in the effort to adopt a well-established risk assessment framework, and the concepts underlying it, to substances that are at least up to a certain level of intake, essential to health.

The risk assessment framework and its underpinnings

A committee of the National Research Council (NRC) published, in 1983, a relatively small report that played a highly significant role in shaping the risk assessment model (1). It defined the relationships between research and risk assessment, and placed upon risk assessment the dual burdens of making sense of sometimes large amounts of diverse and often conflicting data relating to health risks, and of presenting a synthesis of those data in a form useful for practical decision making by risk managers. The committee's by now well-known framework is presented in Figure 1.

An often overlooked but nevertheless critical aspect of the NRC committee's work was the recognition that within the four steps of the risk assessment process, scientific uncertainties arise in several ways and no risk assessment can be completed unless some means for dealing with those uncertainties can be agreed upon. The NRC committee recommended that regulatory agencies in the U.S. specify the ways in which they deal with uncertainties, and that they not deviate from the specified ways unless, in specific cases, scientific evidence is available to eliminate the uncertainty. To deal with uncertainties, agencies have adopted what have come to be called "defaults" and have applied them uniformly unless there are solid scientific data available in specific cases to show that one or more such "defaults" is incorrect (2). Some of the critical defaults used in assessing risks for foreign chemicals are described below.

The NRC committee noted that the choice of defaults was not a purely scientific one, and that elements of "science policy" are unavoidable in any decision regarding the treatment of uncertainty. The committee emphasized the need for clarity and openness in the selection of defaults to avoid the appearance (or reality) of case-by-case manipulation of risk assessment outcomes.

The goal of hazard and dose-response assessment (see Fig. 1) is to estimate a level of chronic daily intake of the foreign chemical that is likely to be without harm to almost all members of the general population. That level [referred to variously as a toxicity reference dose (RfD), an acceptable daily intake (ADI) or a tolerable daily intake (TDI)] is said to represent an approximate threshold dose for a large and diverse human population. The challenge for risk assessment is to provide an estimate of that dose based on data from studies in limited human populations or, far more commonly, from experimental studies. This goal is achieved in the hazard identification and dose-response steps. Human exposure (or intake) assessment is completed, and risk is characterized by a determination of whether and to what extent intakes of the substance exceed the derived estimate of acceptable chronic intake (i.e., the ADI or TDI or RfD).

It should be noted that carcinogenic chemicals are usually treated as if they act through nonthreshold mechanisms, and the approach to assessing risk used for such substances differs from that described here. Carcinogenic risk assessment is not discussed in this paper because it is not likely to be highly relevant to nutrients. Moreover, any nutrients that contribute to cancer risk because of excessive intakes are likely to act through threshold mechanisms.

Hazard identification and dose-response assessment: the experience with foreign chemicals

Following are some of the critical assumptions that underlie hazard identification for foreign chemicals.

1) Hazard identification involves assembling all of the available human and experimental evidence regarding the toxic hazards of a substance and identifying the specific types of harm a substance can cause and the conditions of exposure under which harm is produced.

2) All other factors (e.g., study quality) being equal, the studies demonstrating harm at the lowest doses are typically chosen as the basis for risk assessment.

3) Evidence from human studies, if convincing regarding causation (or at least regarding the strength of the association), is preferred for risk assessment unless its shortcomings (e.g., lack of sufficient exposure duration or lack of quantitative exposure information) are judged excessive.

4) In the absence of adequate data from human studies, data from animal studies are used for risk assessment.

5) As long as the animal data are of high quality, they are selected for use in risk assessment irrespective of whether their specific relevance to humans is known. Only the use of inappropriate exposure routes would make such studies irrelevant.

6) Increasingly various animal models and findings are being investigated for their relevance to humans. Data from such investigations may strengthen or weaken the selection of specific sets of animal data for human risk assessment.

It is often claimed that the use of animal data as the principal basis for estimating human risk does not carry strong scientific support. It is used only because it is rarely possible to develop a convincing and thorough picture of a substance's toxicity in human subjects. Although there are moderately compelling scientific arguments supporting the use of animal data for assessing human risk, there is much to be said for the claim that it is used, at least in part, because there is usually no alternative.

Within the area of dose-response assessment, identification of the levels of exposure (or doses) showing adverse effects and those showing no adverse effects (i.e., no differences from control subjects) are the starting points for deriving a TDI (or the other estimates of population thresholds). The no-observed adverse effect level (NOAEL) and the minimum level showing adverse effects (lowest observed adverse effect level, LOAEL) may derive from studies in humans or in experimental animals. It is at this point that risk assessment encounters the well-established fact of biological variability in response. That such variability in response exists is scientifically certain; it is clear that thresholds vary widely in the general population. The magnitude of this variability is, unfortunately, immeasurable in almost every specific case (2,3).
If the starting point for dose-response assessment is human data, then the problem of biological variability in response concerns the question of the representativeness of the responses observed in the population studied, for the human population to be protected. Specifically, so-called uncertainty factors (UF), typically ranging from three to 10, are divided into the NOAEL derived from the human study used as the starting point for risk assessment. The “reduced NOAEL” becomes the TDI. The use of the UF is aimed at ensuring that the derived TDI represents a “no-risk” intake for the most sensitive members of the general population. A judgment, explicitly described and explained, is used to select the UF in specific cases; if, for example, the study yielding the NOAEL is very likely to represent individuals near the sensitive end of the general population, then a relatively small UF (perhaps, in some unusual circumstances, a UF of one) may be justified. A UF of 10 is generally the maximum chosen for this form of variability (5).

When animal data and NOAEL based on animal data are the starting point for risk assessment, the issues of interspecies and interindividual variabilities in response arise. A UF of 10 is the typical default used, and the animal NOAEL is divided by this factor to derive an approximate threshold dose for the “average” human. A second UF of 10 is used to reduce the “average” human threshold dose further, to yield a TDI. In the case of foreign chemicals these UFs are usually not altered unless chemical-specific data are available to support an alternative (see Human exposure assessment and risk characterization: the experience with foreign chemicals for a discussion of current trends in this area).

Other uncertainties in the data base are common. For many substances data representing chronic exposures are not available, and a UF is used to estimate a chronic NOAEL from a subchronic value. Some studies reveal adverse effects at lowest-observed adverse effect levels (LOAEL), but do not include a dose that turns out to be a NOAEL; again a UF is inserted to estimate a NOAEL. In most cases, for foreign chemicals, total UFs of 100 are used, in a few cases smaller values are used and in a substantial number of cases values greater than 100 are used (5,6).

The bases for these various UFs are found in a somewhat murky interaction of scientific understanding, a public health philosophy of caution and the need for a system that provides, in most cases, usable answers. It is difficult to untangle these various factors, and to attempt to do so here would create an unnecessary distraction. Suffice it to say it is the above-described experience with foreign chemicals that confronted the DRI committee as it undertook its examination of upper levels for nutrients.

Human exposure assessment and risk characterization: the experience with foreign chemicals

Much of the scientific uncertainty associated with hazard identification and dose-response assessment stems from a lack of fundamental knowledge. Assessing exposure to (or intake of) foreign chemicals, including (perhaps even especially) those found in food, suffers in most cases from a lack of data. In addition to the amounts of the substances present in foods, distributions of food intake rates over different populations, over time and place and across genders and life stages, are all necessary to support reliable intake estimates, and all are difficult to acquire in a timely way. The current interest in nutritional and dietary supplements requires similar efforts and the data bases available to support them are relatively meager. Much remains to be done in this area.

Estimates of intake are, nevertheless, made, and regulators tend to focus on “high-end” consumers, individuals at the 90th or 95th percentile of chronic intake. An alternative approach involves the attempt to describe the distribution of intakes across a population, to identify the fraction of the population at potential risk (the fraction having exposures greater than the RfD, ADI or TDI), and to devise risk management strategies to shift the distribution of intakes or at least to eliminate the high (“at risk”) end of the distribution. So-called Monte Carlo simulation methods are now being used to construct such intake distributions and to reduce the weight given to food intake data representing just a few days of consumption (3,4).
Deviations from defaults

During the past 10–15 y a sizeable investment has been made in research directed at learning more about mechanisms of action of foreign chemicals, and the field is growing. Much of the effort has as its goal the development of chemical-specific data that can be used to replace one or more of the typical defaults used in risk assessment. A large share of this effort has been devoted to the study of interspecies and interindividual similarities and differences in pharmacokinetic (PK) models to permit quantitative extrapolations across species without the use of UFs. Less but still significant research effort has been directed at interspecies and interindividual differences and similarities in pharmacokinetic (PK) models that are increasing used to quantify biological variability.

Pharmacokinetics concerns the study of the rates of absorption, distribution, metabolism, and excretion of chemicals; this type of study was first applied to pharmaceutical agents but in recent years efforts to characterize the toxicokinetics of nonpharmaceutical and potentially toxic chemicals have become increasingly common. In the simplest of terms, toxicokinetics attempts to describe the quantitative relationships, over a range of doses, between the amount of chemical entering the body and the amount reaching the cellular site in the body that is the target for toxicity. Differences in these relationships among individuals and between humans and other species appear to account for a large part of the biological variability in response to chemical exposure. Differences among individuals and across species in toxicodynamics, which concerns the nature and degree of cellular response to a given target site dose, probably accounts for any remaining biological variability in response. Developing all the necessary data to understand toxicokinetic and dynamic variability is burdensome, but much progress is being made in developing models from limited data (PB-PK models) that are increasingly used to quantify biological differences and thereby replace default values.

A major and highly creative effort, pioneered by Andrew Renwick, has resulted in a new way of looking at the 10-fold UFs for interspecies and interindividual differences (6). Through a careful analysis of large amounts of empirical data, Renwick was able to “dissect” each factor of 10 into the two types of chemical actions, toxicokinetic and toxicodynamic, that are the principal determinants of variabilities of both types. The result of his work, which has received wide recognition in the risk assessment community, is depicted in Figure 2.

The value of the Renwick work can be seen when, for example, the development of a PB-PK model for interspecies extrapolation removes the necessity for part of the UF of 10, reducing the UF to 2.5 to account for toxicodynamic differences only (Fig. 2). Mechanistic research related to toxicodynamics could replace the UF of 2.5 with actual data on species differences in response to a given dose at target site.

Knowledge of these and other innovations in risk assessments for foreign chemicals provides a useful context from which to approach the problem of health risks from nutrients and supplements.

Nutrients and nutritional supplements: Can the risk assessment framework for foreign chemicals be applied?

The risk assessment framework described in the foregoing is general in nature: it can be applied to any source of risk. Its hallmarks are: 1) a high degree of systematic organization and evaluation; 2) an emphasis on clarity with respect to the bases for all the significant choices made, including the choices of defaults or alternatives to them in specific cases; and 3) the production of results useful for decision making. None of these characteristics is unique to foreign chemicals.

Research on the health effects of nutrients and supplements, however, has not traditionally focused on the same phenomena that have been the objects of investigations in traditional toxicology. The nature of available data on the adverse effects of excess intake is different in many ways from that available for foreign chemicals (not the least of which is that, with few exceptions, there is simply a lot less of it available). And, although issues such as biological variability arise, it is not clear that the specific default assumptions traditionally used for physiologically valueless foreign chemicals are appropriate for substances having defined and often interacting roles in human metabolism and physiology.

Additional questions arise. It is, of course, unethical to subject human beings to excessive levels of nutrients to determine the doses at which adverse health effects develop (although some nutrient trials that examine minor and completely reversible effects are available). Not infrequently, data on adverse side effects are developed during clinical trials, but it is not at all clear how representative such findings are and it is difficult to establish causation. Case reports provide some limited data, but mostly regarding acute exposures. Epidemiology studies are available for many nutrients, but most focus on health benefits; a few, such as those available on selenium, have proved useful but epidemiology studies have not been a major source of information (as they have in the area of foreign chemicals where it has been possible frequently to study occupational cohorts and cohorts of patients on different types of therapy and derive useful hazard and dose-response data). The human data available on adverse effects of nutrient overexposure are not extensive. This can be seen in the several IOM volumes that have emerged from the DRI effort in the past five years.

The IOM search for animal data on this subject turned up fairly extensive data for some substances, particularly the minerals, but little or no published data for most (3). There was little evidence of systematic experimental investigation. Moreover, among those on the IOM committee coming from nutrition backgrounds there was a reluctance to rely upon experimental data as the primary basis for human risk assessment. As noted earlier, experimental data are routinely relied upon for assessing human risk of toxicity from foreign chemicals, but many in the nutrition community remain skeptical of that view for substances having nutritional value, and even for substances such as individual amino acids. This hesitancy probably stems from a belief that any physiological and biochemical difference between experimental animals and humans may create opportunities for nutrient-related derangements in animals that are simply not possible in humans. Another factor causing concern is the well-known practice of using very high doses in animal studies to compensate for the relatively small sample sizes used in most experiments. Use of doses substantially in excess of those that humans will ever encounter may create artifactual responses that have little bearing on human response.

There is, in addition, no agreed upon methodology for applying the risk assessment framework described earlier. Questions regarding the value of experimental data are further burdened with a lack of consensus regarding the use and magnitude of any necessary UFs (3).

It seems clear that much remains to be done before a truly rigorous risk assessment scheme can be established for nutrients and supplements. What will be necessary to achieve this will be
IOM's tolerable upper levels of intake

The upper level (UL), as defined by the IOM, is the highest level of daily chronic nutrient intake that can be consumed by sensitive members of the general population without expectation of adverse effect. As in the case of RfD, TDI and ADI, it is targeted at the sensitive end of the population, and it is clear that many individuals can tolerate levels in excess of the UL without experiencing harm. It is not possible, of course, to know which individuals are more or less sensitive so that risk management decisions focus on controlling exposures at the UL.

As a first step the IOM committee had to develop some broad guidelines on the types of evidence necessary to establish a UL. Four broad categories were developed (Table 1).

For the nutrients falling into category A, it was possible to establish a UL. Decisions regarding the appropriate studies and endpoints tended to emphasize human data, and the UF found to be necessary were generally small, in the range of 2–5, with a few at 10. In most cases the judgments regarding UF selection were based on the view that the adverse effect was relatively minor and reversible upon reduction or cessation of intake, and was also uncovered in studies of individuals already at the sensitive end of the spectrum of sensitivities. Although much remains to be done, the DRI effort is a large step in the direction of scientific rigor.

Macronutrients and amino acids

The most recent effort of the DRI committee involved a look at macronutrients and amino acids. After careful review of a large number of studies the committee found no compelling evidence of adverse health effects from macronutrient excess, so that no ULs were proposed. In several cases, notably those of saturated fatty acids and cholesterol, evidence was found that any increment in intake was associated with an increased risk of coronary heart disease. Any effort to adjust dietary patterns to achieve a UL of zero for those macronutrients would almost surely result in the introduction of different and unpredictable dietary risks. Because of these unusual circumstances no UL was recommended, in this case for substances that clearly cause adverse health effects and for which reliable dose-response data were available (3).

An extensive review of the literature on amino acids had three different outcomes, none leading to the development of a UL. The types of evidence available on amino acids fell into categories B, C or D (Table 1).

As a final point, the absence of ULs for amino acids means that no attempt was made by the DRI committee to provide a detailed evaluation of human exposure to, or intakes of, amino acids through supplement use. The extent and quality

<table>
<thead>
<tr>
<th>Type of evidence</th>
<th>Utility</th>
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<tbody>
<tr>
<td>A. Evidence of harm at some level of intake.</td>
<td>Can be used to derive a UL if it is reliable, relevant and contains quantitative dose-response data.</td>
</tr>
<tr>
<td>B. Evidence of harm in studies of limited or no relevance to humans.</td>
<td>Cannot be used to establish a UL.</td>
</tr>
<tr>
<td>C. No evidence of harm observed in human studies that were designed to detect adverse outcomes.</td>
<td>Cannot be used to derive a UL. Might be used to establish a range of intakes that are likely to be harmless.</td>
</tr>
<tr>
<td>D. No evidence of harm reported in literature, but no scientific studies available.</td>
<td>Cannot establish a UL or any range of harmless intakes.</td>
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of data available on this subject are thus unexamined. It
nevertheless seems important to devote some effort to this
subject because the question of amino acid health risk is likely
to arise in the future, when more and more meaningful health
effects data become available.

**Toward a systematic approach to assessing amino acid risks**

An opportunity exists to shape the future development of
risk assessment for nutrients and nutritional supplements, in
general, and for amino acids in particular. The current
widespread availability of amino acid supplements in some
countries, including the U.S., suggests it is time to focus the
talents of the very finest scientists working in amino acid
chemistry, biochemistry, metabolism, pharmacology and tox-
icology (exactly the type of talent represented at this Hawaii
Workshop) on the problem of assessing potential human health
risk from this highly interesting class of compounds. Such an
effort would, at the least, aim to address two broad questions:

1) What sets of human and experimental studies should be
recommended to provide the most useful and relevant
data to assess any potential risk to human health from
excessive intakes of amino acids?

2) Given the type of information recommended for de-
velopment in (1), how should the risk assessment
framework be applied to define appropriate upper levels
for amino acids (in every case in which available data
suggest that such a UL is needed to protect people from
excess intake).

Although a huge amount is understood about the metabolic
and physiologic effects of amino acids, this knowledge has not
yet been integrated in a way useful for risk assessment. In
addition, the absence of a well-conceived and scientifically
based plan for long-term animal studies of toxicity (including
studies of effects on reproduction and development) remains
a clear gap in the current knowledge base (3,7). Unless the
world's best talent in amino acid science is brought to these
questions, it is likely that regulators will eventually step into the
breach and seek to force upon amino acids the approach to risk
assessment designed to deal with foreign chemicals.

A hitherto unmentioned issue regarding the risk assessment,
risk management framework should not go unnoticed in any
discussion of compounds as biologically active and interesting
as amino acids. To the extent that health benefits of an amino
acid come to be seen as important, and health claims can be
scientifically justified, the amino acid may be evaluated under
the risk management criteria typical of a pharmaceutical agent.
Thus, instead of the "risk-only" decision-making criteria
reserved for foods and dietary supplements (i.e., a risk man-
agement policy that focuses solely on the question of whether
exposures are entirely within safe bounds), such an amino acid
may be judged by taking into account both the benefits it con-
fers and the risks it poses.

Irrespective of the future forms of amino acid regulation,
a scientifically rigorous set of guidelines for obtaining risk-
related data and for evaluating those data for purposes of
assessing human risk and for defining "risk-free" levels would
seem to be essential.

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