The 5th Workshop on the Assessment of Adequate Intake of Dietary Amino Acids: General Discussion 2

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The discussion was chaired jointly by Dr. Ian C. Munro, CanTox, who chaired the U.S. National Academy of Sciences Committee that set the upper levels for nutrients, and Professor Andrew G. Renwick, who served on similar committees in the United Kingdom and within Europe.

In his introduction, Dr. Munro commented that the problems that were raised at the meeting in relation to setting safe upper levels are not unique to sulfur-containing amino acids. The same problems have been faced with many other nutrients, including essentially all of the amino acids. In order to set a safe upper level for sulfur-containing amino acids, it is necessary to integrate data from a variety of different sources, including metabolic data, data gathered from experimental animals including genetically modified mice, data from investigations in humans, and data on inborn errors of metabolism. Such a task is a major undertaking beyond the scope of the present discussion, but a useful outcome would be the identification of further research needs, to assist us in setting safe upper levels for nutrients.

Dr. Munro outlined the basic frameworks that have been used for establishing safe upper levels for nutrients. Figure 1 shows the framework that was elaborated by the National Academy of Sciences in the United States in 1983 and that has been adopted worldwide as the best approach for establishing safe upper levels.

The initial step in toxicological assessment is identifying the various endpoints that are potentially involved, in this case, in sulfur amino acid toxicity and making sure that there are data that cover all of the possible endpoints that would be relevant to the safety evaluation. In many cases hazard identification has to be based on animal studies and in vitro data because in the vast majority of cases we do not have toxicological studies in humans, nor are we likely to get them.

The next step in the process involves hazard characterization, which involves describing as far as is possible the dose–response characteristics. The most important information is the data used to identify the lowest doses at which toxicity first becomes evident and, related to this, to identify doses at which the same toxicity would not be produced when the amino acid was ingested on a chronic basis. Once these intake–response characteristics have been identified for sulfur-containing amino acids, it is possible to develop safe upper intake levels for the general population.

The other equally important part of risk characterization is the intake, which is also known as exposure assessment. Exposure assessment involves determining the exposure to the material of interest, which in the context of this meeting is a sulfur-containing amino acid. For such nutrients the exposure arises from all dietary uses of the material as well as from supplemental use, and it is the total intake that is compared with the upper intake level (UL, see below) to characterize the risk associated with intakes at various levels.

What is necessary for setting safe upper levels is to compile an inventory of all relevant toxicology data and possible adverse effects that have been reported in literature. Often the data will be for effects reported in experimental animals or in vitro systems, and one question that always arises is the relevance of those effects to the in-practice use of amino acids when taken in increased amounts as supplements.

A definition of the upper intake level is “The maximum level of habitual intake from all sources of a nutrient or related substance judged to be unlikely to lead to adverse health effects in humans.” It has also been called the “safe upper intake level” or the “tolerable upper intake level”; whatever the name, risk characterization aims to establish a maximum level of habitual, chronic intake or long-term, from all sources of a nutrient or related substance, that is judged to be unlikely to lead to adverse effects in humans. As part of the definition within the Academy framework, there is an additional important statement, which is that as the intake increases above the upper level, so the risk of adverse effects increases. Therefore, the tolerable upper level, by definition, is not a desirable level of intake, but rather it is a level above which toxicity would begin to be seen.

Professor Renwick highlighted the difficulty of assessment of the massive available database on sulfur amino acids and of assimilating it into an overall picture from which the key areas of toxicological concern can be identified. For each individual amino acid, the key questions are: What is the adverse health effect that is going to drive the risk characterization; in other words, what is the first adverse effect that is detected when intake is increased above normal dietary requirements? Are there sufficient data to describe the intake–response relation?
There is an additional consideration in relation to setting an upper level for nutrients that show metabolic interrelationships, such as the sulfur amino acids. Does the upper level that has been established relate to an individual sulfur amino acid or to the combined intakes of 2 or more sulfur amino acids, in other words a “group upper level”?

For each adverse effect it is necessary to decide: Is it specific to 1 particular sulfur amino acid, or is it related to all sulfur amino acids because they are metabolically interconverted? Are there sufficient data to define the intake–response relationships for each relevant amino acid? How would the intakes of different sulfur amino acids be combined to compare with the “group upper level”?

Information that may help to establish an upper level include metabolic considerations such as: What are the limiting steps in the catabolism of high intakes of amino acid? Is there a biomarker of metabolic overload that can be used to define an excessive intake? Does metabolic overload correlate with the intake–response relation for the toxicity of greatest concern? Is there a biomarker of toxicity we can use to bridge between animal and human studies?

Based on the data presented at this meeting, a final issue for any upper level established for a single sulfur amino acid is the dietary conditions under which it would apply. For example, would the upper level apply only in individuals with adequate intakes of betaine and glycine, or in those with adequate vitamin B status.

The meeting has been presented with huge amounts of data on methionine, limited data on cysteine, and almost no data on cystine or taurine, and the general discussion raised a range of both specific and general issues.

Methionine. It was questioned whether it was sensible to undertake risk characterization for methionine, given the toxicity associated with high intakes. The establishment of an upper level is only one possible output of the process of risk characterization, which could provide useful information by defining the margin of exposure between current dietary levels and those causing adverse effects.

The widely held view that 2 times the intake requirement of methionine is dangerous, which was based on data from studies in rats, were questioned. The human data presented at the meeting suggested as showing that intakes 5-fold or more above the daily requirement are not dangerous. However, it was pointed out that the data were from a small, selected popu-

The steps involved in risk characterization and the formulation of advice for risk managers.

FIGURE 1 The steps involved in risk characterization and the formulation of advice for risk managers.
intakes would not deplete the levels of any cofactor essential to the metabolism of other compounds. Regulation of body load is via reabsorption from the filtrate in the kidneys, and any excessive intake is rapidly excreted in a neutral fashion that does not affect the acid-base balance in the body. Therefore, based on its fate in the body, high dietary intakes of taurine should not be a toxicological problem.

**General issues and conclusions.** There are international differences in the way that supplements are regulated, and that will drive the need for risk assessment. In the United States it is possible to sell a dietary supplement, such as methionine, at some reasonable dose without the need to demonstrate safety at that dose level; it is up to the government to prove that it is unsafe. In other countries, such as Canada, these materials are drugs and not permitted for sale, and a key issue then is demonstration of a clinical benefit.

It was proposed that priority should focus on establishing safe upper levels for cysteine and taurine because they are so widely available to the general population. It was considered that, unlike the other sulfur amino acids, the available data on taurine do not raise significant concerns for adverse health effects, and it is not converted to the other sulfur amino acids, but excess is eliminated unchanged in urine. Therefore, taurine would not be a priority because of health concerns but because of the high intakes that can arise from the intake of some sports drinks.

The issue of how to obtain data from studies in humans was raised. One possibility would be to monitor individuals who use supplement of sulfur amino acids, possibly by measuring the plasma homocysteine before and after taking the supplement. Although it might be difficult to define the toxicological consequences of a rise in plasma level, it would be clear that any increase would not be seen as something good. Another possible source of information is from the clinical use of high levels of supplementation in pediatric patients. A generic problem with the use of such data to set an upper level for any amino acid is that neonates and infants will be synthesizing a lot of protein, which would not occur perhaps in adults. Therefore, the internal dose of free amino acid could be lower in neonates and infants, and they might actually be resistant to any toxicity, compared with an adult.

A problem with reliance on human data, in the absence of knowledge on the mechanism of toxicity, is that human data are generally of short duration. The only way to get long-term data is to perform studies in animals, but then there are the problems of selecting the most relevant species and of extrapolating the data to humans. For example, dietary concentrations of cysteine or cystine that produce death in chicks are far less toxic to rats. Addition of sulfur amino acids to the diet results in rapid depression of food intake and growth suppression. This could be a result of sensory properties, but it was considered to have a physiological basis; it was recognized in the discussion that adaptation occurred to this effect after about 4 days of treatment. The design of animal studies is complex because the sensitivity to amino acid toxicity can vary with the protein content of the diet. The early studies on amino acid toxicity used a low-protein diet to accentuate the toxicity of amino acids under study, but very different results regarding toxicity are obtained for many amino acids when the studies are repeated with adequate protein levels. For example, animals tolerate more methionine with a higher-protein diet than with a lower-protein diet, whereas for cysteine the opposite is true.

The possibility of using the limit of homeostasis as a basis for setting an upper level was discussed. As the body moves from a deficient intake up to an adequate intake, there is an increasing amount of the amino acid in the body. Once the requirement is achieved, there may be no additional retention and no change in body burden if each increment of excess is excreted or metabolized. At some higher level of excess intake above requirement, the body burden may start to increase very steeply; in other words, far more is being taken in than the body is capable of metabolizing and/or excreting. The second breakpoint is important because it was suggested that we can be confident that adverse effects are unlikely below that intake, but the risk of producing adverse effects increases at a higher intake. In other words, an intake below the second breakpoint is likely to be safe. A problem facing application of the approach of metabolic inflection points would be to decide on which is the relevant metabolite to measure. This would be particularly difficult for methionine with its numerous metabolic interrelationships. However, caution should be exercised in relying on toxicokinetic data in the absence of a clear correlation with toxicity because nonsaturating levels of exposure may produce some subtle forms of toxicity on a chronic basis. Therefore, you cannot use those kinetic data alone; you have to use them in conjunction with available safety data to establish a safe upper level. In addition, the complexity of the metabolic interrelationships for methionine means that it would be difficult to define a single toxicokinetic measurement that would adequately reflect an inappropriate metabolic balance within the body.

Metabolic studies with leucine in experimental animals conducted by Ajinomoto have supported the concept of such upper inflection points, based on measurements of the carbon label eliminated in exhaled air. The inflection point is the level at which you start getting a body weight reduction. This type of metabolic limit could be a good predictor of general toxicity, such as weight reduction, in those cases where there is no detectable organ-specific toxicity; because of their generally low toxicity, this may be applicable to many other amino acids. High intakes of methionine produce a specific type of toxicity, hemolytic anemia, reported at this meeting, and also reduce food intake. These effects are probably independent because it is possible to reduce the hemolytic anemia even though the food intake is unchanged. Therefore, it may be possible to dissect out which effects occur at the lowest intakes and then to work out the mechanism and the metabolite(s) responsible for that effect. Once that has been achieved, it would be possible to design suitably focused studies in humans.