Establishing the Upper End of the Range of Adequate and Safe Intakes for Amino Acids: A Toxicologist’s Viewpoint

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ABSTRACT The safety assessment of high intake levels of individual amino acids cannot be based on data from nutritional studies with proteins. Routine toxicity tests designed to investigate a wide range of possible effects should be undertaken for hazard identification and characterization using studies selected to mirror the predicted pattern and duration of human exposure. The approach used to establish an acceptable daily intake level for additives and pesticides, based on defining a “no observed adverse effect” level in the experimental study and dividing by an uncertainty factor that allows for species differences and human variability, has a long history of use for foreign compounds and would provide a suitable basis for determining health-based guidance values for single amino acids. The usual default uncertainty factors for toxicokinetics and toxicodynamics should be replaced by compound-specific values if suitable data are available. In addition, the usual uncertainty factors should be modified to more relevant default values based on species differences and human variability in the biodisposition of amino acids in general or of groups of metabolically interrelated amino acids. There would be no significant health concerns if the human intake levels were below a health-based guidance value developed using this approach. A population-distribution approach could be used to define the magnitude of any risk at intake levels above the guidance value. J. Nutr. 134: 1617S–1624S, 2004.

KEY WORDS: • amino acid • safety assessment • dose response • risk characterization • uncertainty factors • human variability

There is an established procedure for risk assessment of non-nutrient chemicals in food, and considerable experience has been developed over the years by bodies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1). Risk assessment comprises four aspects: hazard identification, hazard characterization (including dose-response assessment) and exposure assessment, which are brought together under risk characterization to provide advice to risk managers (Fig. 1).

A nutrient requirement at normal levels of intake is no assurance of the safety of higher intake levels, and there are well-documented examples of nutrients producing adverse health effects at high intake levels. For example, the safety of high intake levels of vitamins and minerals has been a focus recently of significant risk-assessment activities by national and international bodies such as the Institute of Medicine in the USA (2), the Nordic Council (3), the International Program on Chemical Safety (IPCS) (4), the Scientific Committee on Food (5), and the Expert Group on Vitamins and Minerals in the UK (6).

Hazard identification and characterization define the adverse effect(s) produced by a substance. The nature of the adverse effect determines the approach that is adopted in risk characterization. Different approaches are adopted for genotoxic and nongenotoxic compounds; essential nutrients such as amino acids are generally regarded as nongenotoxic and would not be in vivo mutagens.

A threshold in the dose-response curve is assumed for nongenotoxic compounds, and a point on the dose-response curve that reflects the threshold, such as the “no observed adverse effect” level (NOAEL), is used as the basis for deriving a health-based guidance value such as an acceptable daily intake (ADI). The ADI is calculated as the NOAEL divided by an uncertainty or safety factor (usually 100-fold) (1) (Fig. 2).

The ADI was defined by the JECFA (1) as “an estimate by JECFA of the amount of a food additive, expressed on a body weight basis, that can be consumed daily over a lifetime without appreciable health risk.” Two aspects of this definition require particular attention in relation to the risk assessment of

Fig. 1

Fig. 2

1 Presented at the conference “The Third Workshop on the Assessment of Adequate Intake of Dietary Amino Acids” held October 23–24, 2003 in Nice, France. 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 as a supplement to The Journal of Nutrition. Guest editors for the supplement publication were Vernon R. Young, Dennis M. Bier, Luc Cynober, Yuzo Hayashi, and Motoni Kadowaki.

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3 Abbreviations used: ADI, acceptable daily intake; AD0.5, toxicodynamics uncertainty factor; BMD, benchmark dose; CV, coefficient of variation; ED50, 50% incidence; GSD, geometric standard deviation; HKuf, toxicokinetics uncertainty factor; IPCS, International Program on Chemical Safety; JECFA, Joint FAO/WHO Expert Committee on Food Additives; NOAEL, “no observed adverse effect” level; PBPK, physiologically based pharmacokinetic.
single amino acids that are consumed for a perceived health benefit.

1. The approach adopted for food additives and contaminants is designed to ensure safety across the general population on the assumption that there is no direct benefit to individuals who are exposed. In consequence, the NOAEL and uncertainty-factor approach are sometimes termed “safety assessment” and the associated uncertainty factors termed “safety factors.” Individuals who choose to ingest an increased amount of a nutrient such as an amino acid do so because of potential personal benefits.

2. The ADI is a level of intake that applies to daily intake throughout life and to all life-stages, and in consequence the ADI is usually based on the NOAEL from a chronic study in experimental animals.

Hazard identification for amino acids: the need for data from studies specifically designed to assess toxicity

An extensive battery of testing is required for foreign compounds that may be ingested in appreciable amounts including genotoxicity tests and chronic and reproductive toxicity studies (Table 1). Data from studies on humans may be of particular importance in the risk assessment of amino acids to confirm the absence of reversible adverse effects detected in animal studies (tolerability studies) or to define potential human variability in biodisposition (see below).

The JECFA has evaluated a number of endogenous compounds that are used as food additives, such as citric acid, and recognizes that such substances may require special consideration of both data requirements and the approach to risk assessment of high intake levels. The JECFA guidance on risk assessment, Environmental Health Criteria 70 (EHC 70, ref. 1), states “…the need for extensive testing may be mitigated when the substance occurs naturally in food and has a history of human use or when it is metabolised into normal body constituents.”

Reduced or even minimal toxicity testing can be justified at low intake levels, and EHC 70 (1) states “…if the biochemical evidence shows that the additive makes only a small contribution to existing metabolic pools from food components or in tissues, there may be no need for detailed toxicological studies on it, provided that it conforms to adequate specification.”

Therefore, administration of individual amino acids at low doses, which are consistent with the normal dietary intakes, would not require risk assessment provided that the absorption, metabolism, and disposition values were similar to the same amount consumed as part of a protein.

Although there are data on the adverse health effects of some amino acids (such as neurotoxicity with phenylalanine), these are usually related to a specific endpoint of interest; amino acids have not generally been subjected to open-ended toxicity tests. A major problem faced in setting safe upper levels for vitamins and minerals has been the absence of data from...
Types of toxicity studies necessary for safety testing of food chemicals with high intake levels

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Characterization</th>
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<tbody>
<tr>
<td>Genetic toxicity (genotoxicity)</td>
<td>Various genetic endpoints in bacteria and mammalian cells</td>
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<tr>
<td>Acute toxicity</td>
<td>Used to screen for potential carcinogens</td>
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<tr>
<td>Short-term toxicity</td>
<td>Usually single-dose study</td>
</tr>
<tr>
<td>Subchronic toxicity</td>
<td>Repeated daily doses for 14–28 d</td>
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<tr>
<td>Chronic toxicity</td>
<td>Repeated daily doses for 2 y in rodents</td>
</tr>
<tr>
<td>Reproductive toxicity</td>
<td>Dosing occurs before, during, and after gestation to investigate any effects on fetal or neonatal development</td>
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</tbody>
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1 Additional special studies may be necessary to investigate any effects detected using the standard tests and when there are indications of possible neurotoxicity or immunotoxicity. Data from studies on humans that define absorption, metabolism, and excretion and/or tolerability are also important information (when available).

standard toxicity tests in which all tissues of the body are subject to detailed histological examination (6,7). Many studies investigated the effects of over- or undertreatment with a nutrient on selected parameters that were of interest to the specific researcher. Studies in humans were frequently of little value, either because they did not investigate endpoints relevant to the effects detected in animal studies, or because the primary outcome measures were benefits and the assessment of possible adverse effects was not built into the study protocol.

The safety of nondietary intakes of nutrients can only be assured if testing investigates all possible outcomes, both suspected and not suspected. Screening for possible toxicity will require traditional studies on animals; if human studies are undertaken, these should measure endpoints relevant to the adverse effects detected in animal studies. The problems faced with designing suitable studies on humans are similar to those faced with botanical preparations, because although there may be anecdotal evidence of safety, this may not provide an adequate basis for risk assessment. Ethical considerations and the safety of the volunteers in human studies are of paramount importance, and the primary aims of such studies should be to confirm the predicted safety and tolerability based on other data rather than to investigate the production of adverse effects in humans (8).

Studies to define basic toxicological properties, such as a 90-d study in rodents including reproductive toxicity, would identify target organ toxicity at high doses. The results of chronic animal studies would not be necessary for the safety assessment of short-term intake levels of high doses of amino acids, because amino acids would not cause potentially irreversible effects such as mutations. Therefore, short-term and subchronic repeat-dose studies such as 90-d or 6-mo studies are of greater relevance. An additional reason that chronic studies would be unnecessary is the results of long-term studies in animals are often complicated by the presence of ageing alterations such as nephropathy, which can obscure treatment-related effects and reduce the ability of the study to detect effects other than cancer. It is now widely recognized that studies of 6- and 9-mo duration in rodents and nonrodents are sufficient for identification of possible hazards except cancer and for dose-response assessment of noncancerous effects (9–11).

In addition, special studies may be required depending on the metabolic and physiologic functions of an amino acid; for example, neurotoxicity data would be expected for amino acids that act as or are precursors for neurotransmitters.

There are parallels between consumption of supradietary amounts of amino acids and consumption of increased amounts of botanicals that are normally present in the diet at low levels. In both cases, there can be no presumption of safety at increased levels of intake based simply on the history of prior use. A decision tree was published recently for risk assessment of botanicals and botanical preparations (8) that included 12 different types of information that may be required, depending on the nature of the material and the extent of intake. The types of required information for botanicals were 1) specification of the product; 2) details of the source organism (genus, species, portion of the plant consumed); 3) evidence from previous human exposure; 4) extent of use and estimated intake; 5) technical details of any processing and stability in formulation; 6) nutritional assessment (including assessment of any effects of the active principles on the bioavailability of other dietary components); 7) toxicological assessment (including assessment of any effects of other dietary components on the bioavailability of the active components; there is not a checklist of essential toxicity tests, and the studies performed should be selected on a case-by-case basis); 8) human clinical data including variability of response, adverse-effect reports, and contraindications; and 9) ancillary data (mode of action of beneficial effect to allow consideration of special studies, aggregate exposure, susceptible groups, etc.).

If amino acids are considered using this decision tree, safety data would be required if the proposed use gave a significant increase in intake compared with a high consumer of amino acids (95th percentile) from normal dietary sources; under these circumstances, data would be needed on topics 1, 3, 4, 6, 7, 8, and possibly 9.

An important aspect of amino acids as part of topic 6 above is consideration of the mode of administration, because intake of a single amino acid, not as part of a protein, could alter endogenous body pools of the amino acid per se and/or any of its metabolites, and this could be a toxicological concern. For example, large doses of one amino acid could saturate the transport of other amino acids into the brain and thereby could affect the concentrations of other amino acids and their neurotransmitters. This possibility received considerable attention and stimulated numerous studies and correspondence in relation to phenylalanine, which is formed from the metabolism of the dipeptide sweetener aspartame within the gastrointestinal tract. Wurtman (12) reported that a high single dose of aspartame (200 mg/kg) given to rats with glucose (3 g/kg) increased brain concentrations of phenylalanine and its metabolite tyrosine and decreased brain tryptophan levels. A subsequent paper (13) extended these observations in rats and reported that the same doses altered the concentration ratios of a number of branched-chain amino acids compared with plasma total large neutral amino acids, and that the brain concentrations reflected the plasma ratios. This observation stimulated other studies that also reported changes in plasma or brain concentrations of some amino acids (14) and either reported changes in the concentrations of some neurotransmitters in the brain (15) or did not find alterations in
brain monoamines after single doses of aspartame (16,17) or after chronic treatment (18). Extrapolation of laboratory observations of high-dose aspartame administration in rats, which appeared to be dependent on the animal-dosing regimen, to the risk assessment of aspartame for humans proved to be a particularly contentious issue and stimulated a heated debate (19–23). Despite the inevitable media attention given to studies on aspartame, various observations (reviewed in ref. 24) illustrate that the administration of a source of a single amino acid in isolation rather than as part of a protein can alter the disposition of other amino acids that share common transporters or pathways of metabolism, and this possibility needs to be considered as part of risk assessment.

Hazard characterization for amino acids: dose-response considerations

Application of the risk-assessment procedure that is used to derive an ADI to the safety assessment of high intake levels of amino acids must take into account specific aspects, as follows:

1. There is a normal dietary intake associated with normal function.
2. There are minimum intake levels for some amino acids, which are essential for normal function.
3. There is a potential or perceived health benefit to the individual who chooses to consume high levels, whereas there is no direct health benefit to the individual consumer arising from the presence of non-nutrients in food.
4. The endogenous levels of amino acids are under homeostatic control, so that the concentrations in the body are not related directly to the dose ingested.
5. Amino acids are metabolized by enzymes that show high specificity and sometimes low capacity; these would differ from foreign compound–metabolizing enzymes with respect to species differences and human variability.

To use toxicity data from animal studies for risk assessment of amino acids requires us to define a suitable starting point for hazard characterization and allow for species differences and individual variability in sensitivity between different humans.

Based on experience with aspartame, metabolism and biodisposition studies with single amino acids at doses that are relevant to animal hazard characterization studies and also to predicted human intake levels are critically important in the consideration and interpretation of dose-response relationships (25).

The starting point for establishing a safe or tolerable intake. Traditionally, the starting point for hazard characterization was based simply on the dose-response data for the adverse effect of concern with the identification of a surrogate for the biological threshold such as the NOAEL or benchmark dose (BMD, the dose that yields a predetermined low incidence of an effect, such as a 5% response). The NOAEL was used for >50 years, and it is a readily determined value from most studies. However, it has a number of disadvantages. The main ones are that the value is dependent on the dose levels selected in the study, that the value will be higher for studies of low sensitivity, and that data from doses above the NOAEL are used only to define the nature of the hazard. The NOAEL approach is used to establish an intake with negligible risk such as an ADI but cannot be used to estimate the risk associated with intake levels above the ADI.

In contrast, the BMD is derived by mathematical analysis of the available dose-response data, uses information for all dose levels, and is not dependent on the doses selected (26). An advantage of the BMD approach is that it provides a basis for a more sophisticated analysis of the risks associated with different levels of intake by humans (see below). The BMD approach may be particularly useful for amino acids, because it is possible that any adverse histopathology observations can be linked to a defined perturbation of physiology such as a change in a circulating biomarker, which could be measured also in human studies (27). It is possible that the concentrations of the amino acid per se or an active metabolite may provide a suitable biomarker (27).

Allowing for species differences and human variability. Uncertainty factors are used to allow for species differences and human variability when the NOAEL from an animal study is used as the basis for calculating an ADI. The usual factors are 10-fold for both species differences and human variability. Subdivision of the usual uncertainty factors (28,29) into toxicokinetic and toxicodynamic aspects is important in adapting the traditional approach to amino acids (30).

The concept of subdividing the 10-fold factors was developed to allow relevant quantitative toxicokinetic or toxicodynamic information on the compound under evaluation to replace one of the default factors shown in Figure 3. The types and quality of data suitable for the replacement of one of the default uncertainty subfactors shown in Figure 3 are defined in a series of meetings and are published on the IPCS website (31).

Information on the toxicokinetics of a compound in humans can be derived from direct experimentation in vivo in humans at low doses or by the incorporation of in vitro data into a physiologically based pharmacokinetic (PBPK) model. An advantage of a PBPK model is that the possibility of saturation kinetics in either animals or humans can be incorporated by the use of Michaelis-Menten constants. Information on to-
xicodynamics in humans should be related to the adverse effect of concern produced at doses above the NOAEL, and direct in vivo experimentation is unethical unless a sensitive biomarker for the effect is available (32). Suitably designed in vitro studies using animal and human tissues could provide data suitable for the replacement of the default interspecies uncertainty factor for toxicodynamics (ADUF of 2.5 in Fig. 3) (31). The use of in vitro data can be illustrated by the decarboxylation of levodopa in vitro: renal slices from rats, dogs, and humans showed similar \( K_{\text{m}} \) values, but the \( V_{\text{max}} \) values in the rat and dog were 7.7 and 3.9 mmol.h\(^{-1}\).mg protein\(^{-1}\) compared with a value of 5.8 for human kidney (33). Interspecies factors of 0.8 or 1.5 could be used to replace the interspecies toxicodynamic default (ADUF) of 2.5 in Figure 3 for rats or dogs, respectively, but only if the renal formation of dopamine were the critical and rate-limiting step in the generation of toxicity from levodopa. This example illustrates not only how information could be used, but also the need to understand the mode of action and critical process(es) leading to the generation of the adverse effect of concern for risk assessment.

Replacement of either an interspecies factor or a human-variability factor requires the generation of data for humans. In the case of amino acids, the existing nutritional database can provide useful background information for developing studies and data suitable for risk assessment. For example, the average coefficients of variation for the endogenous plasma concentrations of different amino acids are \(-34\%\) for children and 23% for adolescents (34), but such measurements are complicated by diurnal variations and the effects of recent intakes (35) as well as the nutritional status of the individual. Human variations in the endogenous plasma concentrations of free amino acids reflect interindividual differences in both intake and kinetics (36). In addition, variations in endogenous levels may not reflect intersubject variability in the increase in concentrations arising from the administration of a single amino acid.

The coefficient of variation (CV) for the endogenous plasma levels of homocysteine in a group of 13 healthy adults was 28%, whereas the increase in plasma levels after an oral loading dose showed a CV of 10% (37). The study was repeated in 10 of the subjects and yielded a similar mean estimate with a slightly higher CV (18%); interestingly, there were marked intrasubject variability (Fig. 4), and the same individual did not have the highest plasma level on both occasions. In consequence, the long-term average variability, which would be more relevant to subchronic effects, would be lower than the CV determined on a single occasion. Wide intrasubject variability has been reported for the kinetics of foreign compounds in humans when measured on different occasions (38).

The CV value of 10% for variability in post-dose plasma homocysteine levels could be used (see below and Fig. 6) to replace the default subfactor for human variability in toxicokinetics (HKUF of 3.2 in Fig. 3) provided that plasma homocysteine was the relevant biomarker and the individuals studied adequately reflected the relevant demographic and range of human variability. Measurement of the plasma biomarker in experimental animals under the experimental conditions that gave rise to the NOAEL or BMD and in humans at the maximum predicted intake could be used to replace the interspecies toxicokinetic factor (AKUF of 4.0 in Fig. 3).

In the absence of suitable amino acid–specific data, it may be possible to define more relevant default interspecies toxicokinetic adjustment factors based on the biodisposition of amino acids in general or of groups of interrelated amino acids that share common metabolic pathways such as the sulfur amino acids (39) in test species and humans.

The subdivision of the 10-fold default factors (28) was based on the fate and effects of foreign compounds in humans and animals, particularly therapeutic drugs for which extensive data were available. The extent to which the default uncertainty factors for human variability cover the general population depends on the CV for the relevant parameter (Fig. 5).

Although there are few data on differences between humans in target-organ sensitivity to toxic effects (toxicodynamics) or the fate of toxicants in humans (toxicokinetics), there is an extensive pharmacology database that can be used to analyze the extent of variability with respect to the actions and kinetics of therapeutic drugs. A review of data from clinical studies identified slightly more variability in inherent sensitivity (dynamics) than in kinetics, with average CV values of 51 and 38%, respectively (38). Assuming a log-normal distribution, the 3.16-fold default uncertainty factors for dynamics and kinetics would cover on average 99.17 and 99.91% of the population, respectively. Recent analyses of human variability in the major pathways of foreign-compound metabolism and elimination (40–46) indicate that there is an average CV of 29% in healthy adults for metabolic pathways that do not show polymorphism, 46% for polymorphic pathways, and 33% overall.

In contrast, a CV of 10 (47) or 15% (48,49) was used to reflect human variability in establishing minimum intake requirements for nutrients based on variation in basal metabolic rate, adult protein requirements, and the fact that many biological characteristics have a CV of \(-15\%\) (48). If such a value (10 or 15%) were also applicable to variability in kinetics at high intake levels of amino acids, then the default
uncertainty factor for human variability in kinetics could be modified to provide the same degree of protection that is inherent in the use of the default factor of 3.16 for foreign compounds. Assuming a log-normal distribution and a CV of 33% [based on the average for different pathways analyzed by Dorne et al. (40–46)], the factor of 3.16 for foreign compounds would cover 99.983% of the healthy adult population: the same degree of protection for an amino acid would be given by factors of 1.43 and 1.70 if the relevant CV values were 10 and 15%, respectively (Fig. 6).

The default factors for toxicodynamics (see Fig. 3) would relate to the processes involved in the generation of adverse effects at high doses, and there is no a priori reason to expect that species differences and human variability in sensitivity to the adverse effects of amino acids would differ from effects produced by foreign compounds. Therefore, if the relevant CV was 10 or 15% for kinetics, it would be scientifically logical to replace the overall 10-fold factor for human variability in both kinetic and dynamics by values of 4.5 (1.43 × 3.16) or 5.4 (1.70 × 3.16). Modification of the general 10-fold defaults for amino acids would require a systematic review of species differences and human variability inherent in the biodistribution of amino acids.

Genetic polymorphisms in foreign compound metabolism are not currently taken into account in the uncertainty factors used in risk assessment or the calculation of ADI values. The usual default factors (see Fig. 3) would not be adequate to cover human variability in polymorphic pathways for foreign compounds that are eliminated principally by a single polymorphic pathway such as CYP2D6 (42), CYP2C9 (46), CYP2C19 (44), or N-acetyl transferase (44). Genetic variability that cannot be recognized by the individuals affected must be allowed for in risk assessment and the setting of a health-based guidance value for the general population, because the individuals at increased risk cannot be given specific advice about high intake of the relevant amino acid, for example, phenylalanine from aspartame. Such individuals would not need to be taken into account in establishing a safe upper limit for intake levels of single amino acids. Although most polymorphisms of relevance to the toxic potential of an amino acid may be recognized from their influence on dietary sources, it is theoretically possible that genetic variants that produce more subtle changes in metabolism or transport could only be of significance at elevated, supradietary intake levels. The potential for functional genetic polymorphisms to affect risk at high intake levels should be considered as part of the risk assessment of single amino acid intake levels.

**Risk characterization**

Data on hazard characterization and exposure must be closely interrelated during risk characterization (50). Issues that may be critical, depending on the rationale for the high intake of isolated amino acids and intended pattern of human intake, include the duration of the toxicity study, the mode of administration (such as single-bolus dose once daily or incorporation into the animals' diet), and the life stages investigated. For example, this may mean that certain toxicity studies such as developmental neurotoxicity may be irrelevant if high intake levels of the amino acid were taken only by the elderly, e.g., to prevent Alzheimer's disease.

Establishment of a "safe upper intake level" based on defining a NOAEL and using default uncertainty factors or compound-specific adjustment factors are forms of safety assurance. Based on this approach, there is negligible risk provided that the intake levels are below the safe upper level. However, this provides no information on the magnitude of any risk at the upper level or the consequences of intake levels that exceed the upper level.
An alternative to the normal risk-assessment approach for toxicants was proposed recently whereby a population-distribution model was used to estimate the risk associated with exposure to compounds producing threshold effects (7). In this method, a well-characterized point on the dose-incidence curve is used as the starting point rather than the NOAEL. The starting point may be derived from analysis of comprehensive dose-response data in different individuals (see ref. 26 for estimation of what is termed a critical effect size) or from a study in humans in which the incidence of a change has been defined. If the incidence of the change was established in a study on animals, the dose for animals could be converted to a human-equivalent dose by dividing the usual 10-fold uncertainty factor or by a compound-specific factor (see above). An advantage of the proposed method is that physiologically impossible results are not generated, because the incidence of a predefined magnitude of response such as a specific change in a serum biomarker is modeled rather than the magnitude of the response. The method can define the incidence of the predefined response at doses other than the starting point, based solely on the relevant CV for human variability in response. Data on actual human variability in sensitivity to foreign compounds is not known, and a default CV would need to be used. Based on the variability in dynamics and kinetics of therapeutic drugs, a CV of 40% was used to illustrate the approach (7). In the case of amino acids, a lower default CV to cover both dynamic and kinetic variability could be appropriate, provided that the value was substantiated by a comprehensive review of relevant published data.

The approach is illustrated in Figure 7, in which a 40-mg dose in humans gave a 5% incidence of a defined biological change, and overall CV values of 40 and 15% were used to extrapolate to the incidences at other doses. The percent incidence is extrapolated from the data point (in this case, 5%) to a 50% incidence (ED50) using the geometric standard deviations for CV values of 40 and 15% and the NORMSINV function on Excel (see references 7 and 42 for additional explanation). The incidence of 5% at a 40-mg dose is converted to a 50% incidence using a log-normal assumption with coefficients of variation of 40 or 15% (for this example), which are equivalent to geometric standard deviations (GSDs) of 1.470 or 1.161 (see reference 42). The log of the dose adjustment necessary to move from the nth percentile (e.g., the 5th percentile) to the median value is given by multiplying the log-GSD by the NORMSINV value for that percentile (which is −1.645 for the 5th percentile). The log-GSD values for 40 and 15% are 0.1673 and 0.0648, respectively, so that the log-GSD multiplied by the NORMSINV values are −0.2752 and −0.1066, respectively. The ratios of the percentile to the median are given by 10 raised to the power of the product of the log-GSD and NORMSINV for that percentile, which in these examples are 10−0.2752 and 10−0.1066, respectively, or 0.5306 and 0.7823. An ED50 of 75.4 mg (40 mg/0.5306) would give an incidence of 5% at 40 mg if the CV were 40%, whereas an ED50 of 51.1 (40 mg/0.7823) would give an incidence of 5% at 40 mg if the CV were 15%. The incidence for any dose can be calculated from the ED50 and the appropriate CV using the NORMSINV function.

A further development of the approach described by Renwick (7) is currently under consideration by the ILSI Europe Expert Group on Risk-Benefit Analysis for Nutrients Added to Foods in which the approach is extended to consider the balance of risks associated with both low intake (deficiency or the absence of a benefit) and high intake (the generation of toxicity) levels. Such a risk-benefit approach, or more accurately a risk-risk comparison, should provide a useful method for risk managers to weigh the potentially adverse effects of not allowing supradietary intake levels of single amino acids against the adverse effects of excessive intakes.

LITERATURE CITED


FIGURE 7 Dose-incidence modeling for hypothetical compounds producing a 5% incidence of a predefined change at a dose of 40 mg, assuming a CV of 40% for a foreign compound and 15% for an amino acid. (See references 7 and 42 for details of calculations.)