Improving Regulatory Decisions through Targeted Research: A Case Study Concerning Amino Acids

Michael D. Rogers* and Miro Smriga**

This paper argues that the EU regulatory practice in the food area may be unnecessarily applying the Precautionary Principle by focussing on upper intake limits for naturally occurring nutrients, while not controlling the quality of the ingredients used in commercial products even though precedents of public health issues arising from adulterated ingredients do exist. Risk governance depends heavily on expert evidence and the case of amino acid supplements is used to document an industry-supported effort to strengthen the science database and thus enhance the regulatory process: Thus ensuring that amino acid use in the EU is safely and proportionately regulated. Scientific work conducted in the last decade by the not-for-profit association, the International Council on Amino Acid Science (ICAAS) is used as a simple case study highlighting the role of proactive clinical research in an era characterized by precaution in risk management, and by the escalating costs of scientific research, and the growing influence of the internet.

I. Introduction

Regulators may pretend, and the general public might believe, that regulations concerned with the governance of risk are introduced in an orderly manner in response to well established needs. However, such an “orderly manner” rarely exists. Harold Macmillan, the UK Prime Minister from 1957–1963, when asked by a young journalist (after a long dinner) what was most likely to blow governments off course replied: “Events dear boy. Events”. One such “event” which ultimately completely changed the landscape of food risk regulation in the EU was the arrival of Mad Cow disease (or Bovine Spongiform Encephalopathy, BSE) in the UK.

The first case of BSE in the UK occurred in 1985, followed by rapidly increasing numbers of BSE-infected cattle across England. The UK epidemic peaked in January 1993, with 1000 new cases diagnosed per week. It then fell fairly quickly to fewer than 200 cases per week in 1997. However, in 1996 the UK government announced that a new form of Creutzfeldt–Jakob disease (vCJD) had appeared and that the likely vector was the eating of BSE infected beef.

This “event” and the accompanying public outcry eroded the reputation of science concerning food safety and nutrition. It ensured that food safety regulations in Europe shifted from being based on well-established risk analyses (based on “sound science”) to much more precautionary regulations (from “modern” to “post-modern” regulations)\(^3\), even if the need for scientific advice remains very high for precaution-based regulations\(^4\). The complexity facing “post-
BSE” regulators is not only in balancing science and precaution, but also in considering a growing range of non-rigorous but influential Internet resources. Moreover, the cost burden of regulatory practice is rising significantly for precaution-based regulations with the REACH Regulation (Reg. 1907/2006 EC) being an appropriate example. Consequently, the freedom to impartially reflect scientific advice is compromised more than ever before. Yet, even at this moment, few would dispute that expert advice should be received and considered by the regulator before a regulatory decision is taken. As Bernal wrote as early as 1944, “The scientist does not claim or want to be on top, but it is not nearly enough to have him merely on tap. In most cases the executive authority will not be able to see for himself when the scientist should be called in or what questions he should be asked”.

In this article, we highlight the proactive and targeted scientific process organized by a not-for-profit organization and propose that an interaction of regulatory bodies with academia and scientific organizations may be the key to shaping the regulatory process in an environment characterized by (A) the escalating costs of science, (B) precaution in risk management and (C) internet penetration. We use the specific example of the International Council on Amino Acid Science (ICAAS) which was created in the early 2000s with the objective of working on science and expert advice in order to ensure that actions taken concerning amino acids under the new food safety regulatory framework were sound. ICAAS is an international not-for-profit organisation, which is registered in Belgium. It has the primary objective of exploring and resolving scientific issues related to all matters concerning the safety, quality and use of amino acids, with particular emphasis on the dietary use for humans. Since its foundation, ICAAS has spent around 3 million US dollars on amino acid safety research projects and funded and organised eight amino acid assessment workshops (Table 1). Furthermore, it has gained substantial backing from the leading academics in the field and accumulated significant know-how and built up a database on amino acid safety. This article examines the European regulatory situation from the ICAAS perspective i.e. focussing on the use and safety evaluations of amino acids in foods and supplements. Because amino acids are less frequently found in supplements in Europe, compared to the USA or Brazil, for example, and are less extensively regulated, compared to vitamins or minerals, they provide a useful and forward-looking case study.

II. Post-BSE Food Safety Regulations in the EU: Where do Amino Acids Fit In?

Before proceeding to the specific regulations that apply to amino acids and the safety issues of amino acid applications in the EU, we have to be clear about the regulatory distinctions concerning “ingredients” and “additives”, as follows:

**INGREDIENTS (I):** Ingredients have either a nutritional function or are added to food for “non-technological” purposes. A good example is the essential amino acid lysine or vitamin C, which can be an ingredient (labelled vitamin C) or a technical additive (labelled with a corresponding E-number) and this distinction depends on its final function in the food (if its function is acid regulator then it is an additive, if its function is to combat vitamin C deficiency then it is an ingredient).

**INGREDIENTS (II):** Particular nutritional uses, or "PARNUTS" (Directive 2009/39), are defined as follows “Foodstuffs for particular nutritional uses are foodstuffs which, owing to their special composition or manufacturing process, are clearly distinguishable from foodstuffs for normal consumption, which are suitable for their claimed nutritional purposes and which are marketed in such a way as to indicate such suitability”. This Directive often concerns amino acids (the coverage extends from baby foods, to elderly diets, to special diets for medical purposes, to sports foods, and a separate Directive may be issued for each category of usage). The continued existence of

---

5 Michael Specter, Denialism: How irrational thinking hinders scientific progress, harms the planet and threatens our lives (London: Duckworth Overlook, 2010).


8 The references to “on top” and “on tap” in this quotation relate to one of Winston Churchill’s dictums namely that “Scientists should be on top not on tap”.

9 Further information is available on the ICAAS website, available on the Internet at <http://www.icaas-org.com/> (last accessed on 13 April 2012).
the PARNUTS Directive remains questionable due to the new framework for health claims on foods and supplements.

**ADDITIVES:** Additives are a special category of “ingredients” that have pre-defined technological functions in the final food. Only two amino acids are categorised as additives in Europe (glutamates and cysteine). All other amino acids are INGREDIENTS within the above meanings. This paper deals exclusively with the non-additive use of amino acids.

With these distinctions in mind, the following regulations are applicable to non-additive ingredients:

1. **The Food Additions (Ingredients) Regulation** (1925/2006) which is also primarily aimed at vitamins and minerals but other additives may be added: Article 5.3 states “upper safe levels of vitamins and minerals established by scientific risk assessment based on generally acceptable scientific data”. Article 8.2(b) states “If the possibility of harmful effects on health is identified but scientific uncertainty persists, the substance shall be placed in Annex III, Part C”. NB Changes to the Lists are governed by Regulation 108/2008.

2. **The Food Supplements Directive** (2002/46) which is currently aimed primarily at vitamins and minerals but in principle other supplements are to be considered later (Article 4.8 which should impact on all micro-nutrients). (See also Preamble paragraph 6 “There is a wide range of nutrients and other ingredients that might be present in food supplements including, but not limited to, vitamins, minerals, amino acids, essential fatty acids, fibre and various plants and herbal extracts”.

3. **The Nutritional Health Claims Regulation** (1924/2006): “Health claim means any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health” (Article 2.5). “There is a wide range of nutrients and other substances including, but not limited to, vitamins, minerals including trace elements, amino-acids, essential fatty acids, fibre, various plants and herbal extracts with a nutritional or physiological effect that might be present in a food and be the subject of a claim” (Preamble paragraph 9). There is a reversal of the “burden of proof” from the regulator to the producer so the Regulation will tend to deal first with substances that are well evaluated in terms of safety (vitamins and minerals). See also Preamble paragraph (25) “Health claims other than those referring to the reduction of disease risk, based on generally accepted scientific data, should undergo a different type of assessment and authorisation. It is therefore necessary to adopt a Community list of such permitted claims after consulting the European Food Safety Authority”; and paragraph (29) “In some cases, scientific risk assessment alone cannot provide all the information on which a risk management decision should be based. Other legitimate factors relevant to the matter under consideration should therefore be taken into account”.

Since neither the purity nor the upper limits of amino acid use are specifically regulated when used for nutritional (non-technological) purposes, they are subject to general EU Food Law, national laws in each member state and to the mutual recognition principles (i.e. national rules must be necessary, proportionate and non-discriminatory and not create obstacles to trade). Discrepancies among the Member States exist: For example, while the UK and Ireland allow amino acids in foods and supplements without prior notification, the Netherlands stipulates certain maximum levels and requires pre-marketing notification. Many other Member States do not have specific rules for amino acid products and generally require pre-marketing notifications on each individual product. The regulation of vitamins and minerals (Reg. 1925/2006) described above suggests that, once a regulatory framework is established for amino acids, the EU authorities may well focus the thrust of their efforts towards limiting maximum levels of amino acids rather than concentrating on quality-related aspects of their use.

The current absence of an EU regulatory framework means that three risks can be identified for this specific group of nutrients: (1) Products with excessive levels of amino acids that may endanger health if used by certain groups of consumers over prolonged periods; (2) Low level intakes of nutritionally essential amino acids in specific populations; (3) Use of low quality products with high levels of impurities or heavy metals. ICAAS has been closely involved in elaborating new approaches to all three of these problems, as outlined in the next section.

---

10 See the Consolidated Version of the Treaty on the Functioning of the European Union, Official Journal 2008 (115/47) in particular Articles 34 and 36, Article 114 and Article 191.2
III. Risk Assessment and Amino Acids

The twenty amino acids that make up proteins are the oldest nutrients on Earth and provide minimal requirements for growth, nitrogen equilibrium, maintenance of host defences, neural and muscular functions, and gene expression regulation. The catabolism of amino acids is an important source of energy. The mammalian body is incapable of storing large amounts of amino acids and their homeostasis must be finely maintained through food intake and an orchestrated action of organs and tissues. Nine of the twenty amino acids are considered nutritionally essential, which means they should be ingested daily in a good diet. Three other amino acids (namely, arginine, glutamine and glutamate) have been documented as semi-essential, which means that they become essential in specific groups of people in specific situations, for example during major stress or infection. While the science of amino acid effectiveness in disease and health is evolving, controversies remain (e.g., the effectiveness of branched-chain amino acids to combat muscle mass loss in the elderly). Therefore, this paper deals only with the toxicological and regulatory aspects of the dietary use of amino acids.

1. The situation when “too much” of an amino acid is ingested: For amino acids, similarly to vitamins and some other micro-nutrients naturally present in food and human body, the general shape of the risk assessment relationship shows harms at both low intakes (inadequacy) and at high intakes (excess). The harms associated with excess intake of amino acids are generally less frequent and serious than the harms associated with insufficient intakes. Yet, much regulatory effort has been devoted to establishing upper limits for naturally occurring nutrients, as outlined in Section 2 of this paper, especially for the case of vitamins and minerals.

For amino acids in foods and supplements such an effort was conducted mainly in the USA and Japan in the early 2000s. This effort was triggered by the widespread use of amino acids in supplements and foods in both countries. The relevant authorities came to the broad conclusion that for commercially used amino acids such limits were not yet necessary and they recognized that there wasn’t a sufficient database to make informed decisions, and that the financial resources needed to answer such questions would have been substantial. The cost problem stems mainly from the fact that the traditional toxicological approaches do not resolve the problem of upper limits for naturally occurring nutrients present in food and new approaches are needed. This is best illustrated by well-established rodent sub-chronic (13-week-long) studies that determined the “no-observed adverse effect levels” (NOAEL) for six amino acids. Surprisingly, or perhaps not surprisingly, those levels were below the levels of amino acids normally ingested in food!

Thus, in the early 2000s, the stakeholders in academia and industry knew that upper levels for amino acids might be needed in the near future, but did not know how to determine them, especially since the authorities did not have the resources to underwrite the necessary research. At that moment, the industry-supported ICAAS stepped in and pro-actively decided to pool and invest financial resources into academic research and to establish framework(s) in order to evaluate upper limits for amino acids – at least for those that were frequently used by the general population (i.e., leucine, isoleucine, valine, arginine, tryptophan, lysine, methionine and glutamine). The greatest advances so far reported from this effort are for the amino acid leucine, which is often taken at high doses by athletes.


An ICAAS-supported rodent study\textsuperscript{16} studied the effect of chronic excessive leucine intake (up to 8% (w/w) in a diet) in rats by making use of transcriptomics and metabolomics. Summarized data indicated that a leucine intake of up to 8% of the diet showed no adverse effects when moderate or high amounts of protein were consumed. But, if rats consumed a diet with extremely low levels of protein, the gene-marker panel suggested that dietary leucine supplementation of 2% might be the maximum tolerable dose. Transcriptome and metabolome analyses indicated that amino acid metabolism in the liver was drastically affected by excessive leucine, but only in the case that rats consumed very little protein. From DNA microarray analysis of the liver of low protein groups, several genes relevant to growth or the cell cycle were changed.

An innovative human study supported by ICAAS, which was conducted in Canada, determined the metabolic and adverse effects of the acute ingestion of very high intakes\textsuperscript{17}. Healthy adults received graded stepwise increases in leucine intakes of 50, 150, 250, 500, 750, 1000 and 1250 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}. Leucine oxidation was determined using L-[\textsuperscript{13C}]Leucine and the appearance of 13CO\textsubscript{2} (F\textsubscript{13CO\textsubscript{2}}) in the subject’s breath. A range of markers were used to monitor for adverse effects including glucose, insulin, ALT and ammonia. Indeed, an increase in blood ammonia concentrations were observed at leucine intakes higher than 500 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}. There were no changes in liver transaminases. Glucose levels fell significantly but within the normal range and without any change in insulin. This innovative acute study is the first to directly estimate the safe upper limit of leucine intake in humans. Ammonia observations raise concerns that intakes greater than 550 mg/kg/d or 39 g/d may be a potential, if not serious, risk to health. This dose is 7–8 times higher than the average intake of leucine in a typical human diet (unpublished results), creating a significant safety margin for its use in supplements in athletes and/or elderly populations. The above animal study implies, however, that in people with extremely low protein intake, the toxicity of amino acids might be different than that in the normal population. To the best of our knowledge, the above work by Elango and colleagues\textsuperscript{18} is not only innovative, ground-breaking research that directly points to a potential upper limit for a frequently used amino acid, it is also an illustration of a commercially-driven and a pro-active scientific approach that should be used by regulators in the future more widely. The interest of responsible industry to invest in science and determine upper limits of nutrients before the authorities do so is based on precaution, the health concern of the final consumer to eat safe food and the interest of the regulator to make clear and informed decisions converge in a straightforward “win-win-win” manner.

The European Commission’s Communication on the Precautionary Principle\textsuperscript{19} requires that precautionary risk management action should be subject to proportionality and cost benefit criteria\textsuperscript{20}. Applying the above “ICAAS approach” to studying amino acids in humans, such as leucine; one may conclude that precautionary risk management actions relating to amino acids would not be justified on either proportionality or cost-benefit grounds.

2. The situation when “too little” of an essential amino acid is ingested or the protein intake is of low quality: This is the second area of concern. Dietary protein and amino acid requirement recommendations for normal “healthy” children and adults have varied considerably over the last 4 decades. The latest FAO/WHO protein requirement estimates\textsuperscript{21} for children were decreased, but essential amino acid requirements for adults were more than doubled when compared to previous recommendations – reflecting the important role of amino acid balance. Amino acid fortification today is not only about improving protein quality\textsuperscript{22}. Recent science indicates that nutritional requirements should take into account common living conditions such as energy deficit, infection burden, vegetarian diet, severe stress, gastrointestinal health and other fundamental demands.


\textsuperscript{18}Ibid.


\textsuperscript{20}Ibid.


3. The problem of quality control

ICAAS has devoted substantial attention to the potential health risk of ingesting amino acids of inferior quality. This was triggered, among other incidents, by the 1989 disaster caused by an impure product containing an amino acid tryptophan which caused so-called Eosinophilia Myalgia Syndrome (EMS) and consequently several deaths in the USA. Over a period of months, the Centres for Disease Control identified 1531 EMS cases in the US population. Given the seriousness of the condition, the US Food and Drug Administration subsequently banned supplement use of tryptophan. Once the ban was in place, EMS occurrence dropped to zero. EMS symptoms were a high peripheral eosinophil count and disabling myalgias which have never been reported with the intakes of high quality tryptophan – even at very high doses. It also became evident that almost all EMS cases were traceable to a product made by a single company and that cases of EMS were not associated with the use of tryptophan from other manufacturers. A contaminant in tryptophan, rather than tryptophan itself, was therefore considered the likely cause. It is not yet fully known which contaminant was the culprit, but 3-(phenylamino)alanine (PAA) received most attention. One has to note that the tryptophan product which triggered EMS in 1989 fully satisfied the existing specifications yet killed tens of people and that PAA was not and is not included in the EU or US Pharmacopoeia or Food Chemical Codex standards! We argue that in this case, applying the precautionary principle would be both prudent and justified. A counterargument is that the analysis of PAA presence in nutrients is technically complicated and costly and that classical HPLC techniques might not be sufficient to detect it. However, internal ICAAS data (Fig. 1) show that the currently used supplements have relatively high levels of undisclosed impurities that are readily detectable by classical HPLC. As a precautionary approach the Pharmacopoeia purity level or a certain cut-off level for "summarized impurities" (Fig. 1) would be an appropriate threshold for potentially "most risky" samples entering the supplement market. With regret, ICAAS notes that at this time, no specifications for the use of amino acids exist in the EU and while precaution is often unnecessarily applied to upper limits on nutrients that are perfectly safe, no such an approach is used when proven risk factors such as quality and specifications are concerned. Unfortunately, the experience with vitamins and minerals (Section 2) indicates that this aspect of consumer protection might recur as vitamin/mineral specifications are guided only by the Food Chemical Codex.

To counter this challenge, ICAAS proactively works on a global level, discussing with the U.S. Pharmacopoeia on adjusting the Food Chemical Codex specifications for amino acids as early as 2012. The purpose of the adjustment would be to eliminate the most severely adulterated amino acid supplements from the markets worldwide by using an ICAAS-invented and simple-to-conduct analytical method.


IV. Expert Advice and Smart Regulations

This article outlines a single case of amino acids within the context of the existing legislation on vitamins and minerals. As such, this particular case cannot be generalised; and the authors recognise the EU Commission’s attempts to improve risk assessments across a broad range of products. As part of the Commission’s programme to produce a Governance White Paper\(^26\), a Working Group on “Democratising Expertise and Establishing European Scientific References” was created with the following mandate: “To formulate proposals to improve the contribution of expertise to European governance, taking into account its increasing involvement in risk management and its linkages with all levels (sub-national, national, international). This involves a better understanding of the sources and functioning of expertise itself, and of its use for public policy and societal debate in areas involving uncertainty and risk”.\(^27\) Following on from this working group’s report the Commission issued a Communication on the “Collection and Use of Expertise by the Commission: Principles and Guidelines”\(^28\). This Communication stressed three core principles for selecting expertise, namely, Quality, Openness and Effectiveness. The clear implication is that for expert advice to be effective it has to be the best and most comprehensive expert advice and not just that provided by closed committees. Only then will the current drive for Smart Regulations\(^29\) be achieved.

Since the amino acids regulatory framework is still to be created; the safety lessons from outside the EU, as well as the experience with vitamins and minerals, could be applied – perhaps in the way the authors attempt to illustrate in this paper. By way of conclusion; we all want to improve risk regulation and thus call for regulators to make better use of the expert communities. Experts don’t want to be on “top”, but do want to be heard\(^30\) and to make a useful contribution to the regulatory process. Real expertise is a scarce commodity and procedures have to be in place to ensure that it contributes to “smarter” regulations.


\(^{30}\) John D. Bernal, Lessons of the war for science, supra note 7.
**Table 1.** A short summary of the eight ICAAS-sponsored amino acid assessment workshops that involved the leading academic scientists and regulators from more than 15 countries.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date</th>
<th>Place</th>
<th>Targeted amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>June 2001</td>
<td>Tokyo</td>
<td>Evaluation Methods</td>
</tr>
<tr>
<td>2nd</td>
<td>Oct. 2002</td>
<td>Hawaii</td>
<td>Evaluation Methods</td>
</tr>
<tr>
<td>3rd</td>
<td>Oct. 2003</td>
<td>Nice</td>
<td>Evaluation Methods</td>
</tr>
<tr>
<td>4th</td>
<td>Oct. 2004</td>
<td>Kobe</td>
<td>Leucine, Isoleucine, Valine</td>
</tr>
<tr>
<td>5th</td>
<td>Oct. 2005</td>
<td>Los Angeles</td>
<td>Cysteine, Methionine</td>
</tr>
<tr>
<td>6th</td>
<td>Nov. 2006</td>
<td>Budapest</td>
<td>Lysine, Arginine</td>
</tr>
<tr>
<td>7th</td>
<td>Nov. 2007</td>
<td>Tokyo</td>
<td>Glutamine, Proline</td>
</tr>
<tr>
<td>8th</td>
<td>Nov. 2011</td>
<td>Washington DC</td>
<td>Leucine, Tryptophan</td>
</tr>
</tbody>
</table>

**Fig. 1.** HPLC profiles of the amino acid tryptophan (Trp). Panel A depicts a typical pharmaceutical grade Trp. Panel B1 and B2 show HPLC profiles of randomly sampled supplements. Small peaks before and after the large Trp peak are undisclosed impurities. The level of impurities eluded before/after Trp were summarized and quantified in terms of “particle-per-million” (ppm). Note the substantial difference in purity between the pharmaceutical grade (panel A) and supplements (panels B, internal ICAAS data).