Risk Assessment Paradigm for Glutamate

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Abstract

Background: Re-evaluation of the use of glutamic acid and glutamate salts (referred to as glutamate hereafter) by the European Food Safety Authority (EFSA) proposed a group acceptable daily intake (ADI) of 30 mg/kg body weight (bw)/day. **Summary:** This ADI is below the normal dietary intake, while even intake of free glutamate by breast-fed babies can be above this ADI. In addition, the pre-natal developmental toxicity study selected by EFSA, has never been used by regulatory authorities worldwide for the safety assessment of glutamate despite it being available for nearly 40 years. Also, the EFSA ignored that toxicokinetic data provide support for eliminating the use of an uncertainty factor for interspecies differences in kinetics. **Key Messages:** A 3-generation reproductive toxicity study in mice that includes extensive brain histopathology, provides a better point of departure showing no effects up to the highest dose tested of 6,000 mg/kg bw/day. Furthermore, kinetic data support use of a compound-specific uncertainty factor of 25 instead of 100. Thus,

an ADI of at least 240 mg/kg bw/day would be indicated. In fact, there is no compelling evidence to indicate that the previous ADI of “not specified” warrants any change.

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Introduction

For food additives, it is customary for regulatory authorities to establish an acceptable daily intake (ADI) value. Traditionally, this is done through identification of a no-observed-adverse-effect level (NOAEL) or a benchmark dose lower confidence limit (BMDL) from a suitable key study, usually a 90-day rodent or long-term carcinogenicity/toxicity study, to which appropriate “safety” or “uncertainty” factors are applied. In the case of a NOAEL or BMDL derived from such a subchronic/chronic rodent toxicity study, usually a 100-fold default uncertainty factor is applied, consisting of 2 times a factor 10 to account for each of interspecies variation and inter-individual variation. This calculated value then represents the ADI, the amount that persons over the age of 12 weeks could consume every day over a lifetime without

expectation of adverse effects. For food additives, provided they test negative for genotoxicity, this risk assessment paradigm has been in place for more than 50 years.

While the NOAEL/BMDL-safety/uncertainty factor approach was developed for and has served the public well in terms of deriving a safe level of use for food additives, the paradigm is not amenable to the risk assessment of nutrients or other major macronutrients such as fats, carbohydrates, proteins, and individual amino acids. Daily intakes of these substances in the human population at levels required for normal physiological function are in grams or tens of grams daily quantities. As a result, it is generally not possible to demonstrate the existence of a 100-fold safety factor for human intakes. Attempts to dose laboratory animals at levels that could approach 100-fold of such normal human dietary intake levels result in nutritional and metabolic imbalances that often yield secondary adverse effects, including weight loss and increased mortality [1].

Recently, the pitfalls of attempting to apply the food additive NOAEL/BMDL-safety/uncertainty factor paradigm to macronutrients has been highlighted in the re-evaluation of glutamate by the European Food Safety Authority (EFSA) [2]. The EFSA established a group ADI of 30 mg/kg body weight (bw)/day based on a neurobehavioral toxicity study [3] in which a NOAEL of 3,200 mg/kg bw/day was identified, to which was applied the default 100-fold uncertainty factor. Previously, the ADI for these substances was set as “not specified” by both the Scientific Committee on Food (SCF) [4] and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [5–7]. JECFA previously considered glutamate and related substances as macronutrients and noted that there were no safety concerns at current levels of intake. The ADI of 30 mg/kg bw/day is at a level below the normal dietary intake of these substances and is in contrast to the results of previous evaluations of the safety of glutamate [5–8] and calls into question the safety evaluation process for a non-classical food additive.

The EFSA evaluation is also unique in that the study selected by the EFSA on which to base the ADI, a prenatal developmental toxicity study [3], has never been used by regulatory authorities worldwide for the safety assessment of glutamate despite it being available for nearly 40 years. The present paper, therefore, aims to re-analyze the food additive risk assessment paradigm performed by the EFSA for glutamate within the framework of the available data, including data on toxicokinetics, data on the (lack of) toxicity in experimental animal studies including the study reported by Vorhees et al. [3], the

use of a (compound-specific) uncertainty factor, and the estimated daily intakes of E620–E625 from a regular diet. This evaluation was brought to bear while understanding that a classical risk assessment for a food additive is not a customary approach for a macronutrient.

Results

Toxicokinetics

The systemic availability of orally administered glutamate is known to be very low, even following large doses. This is due to the extensive metabolism in the gut where glutamate is utilized as a substrate for energy production by enterocytes of the small intestine, thus limiting its absorption into the blood stream [9–13]. Glutamate concentrations in plasma rise significantly only under scenarios of intubation of bolus doses of 150–2,000 mg/kg bw administered in water [14, 15]. When mixed with diet, much lower plasma peak concentrations are achieved in relation to bolus dosing. In a series of studies conducted with human volunteers, Stegink et al. [15–17] demonstrated that oral glutamate exposures of 150 mg/kg bw as a bolus dose in the presence of dietary constituents, notably carbohydrates, produce only modest increases (up to 2-fold) in plasma glutamate concentrations. The rate of utilization of glutamate and its use as an energy source is further enhanced by the presence of carbohydrate in the diet [15–18], as would occur with the consumption of foods containing glutamate.

The lack of significant increase in plasma concentrations (except at excessive bolus doses that cannot be achieved by dietary intake in humans), is in a large part due to the use of glutamate as an energy source by the intestinal cells immediately upon absorption [19]. Peng et al. [20] reported that the feeding of MSG at 5% in the diet (~2,500 mg/kg bw) (forced feeding) of adult rats resulted in only a 2-fold elevation of plasma glutamate. This compares to increases of 5- to 11-fold when rats were administered 1,000 or 4,000 mg/kg bw as an oral bolus dose [21, 22].

Tung and Tung [23] demonstrated that when normal Chinese adults (7 male and 7 female subjects) consumed 150 mg MSG/kg bw as part of a typical Chinese meal (rice porridge), the mean serum glutamate rose to approximately 1.3-fold over baseline 15–60 min after meal consumption. Tung and Tung [23] further evaluated the toxicokinetics of glutamate resulting from MSG consumption through infant formula. Eleven (6 male and 5 female) healthy term infants and 2 preterm infants (1 of each sex) who had received milk about 3 h prior, were fed 20 mL of

an infant formula providing 150 mg MSG/kg bw. The formula was a powdered milk-based product with added sucrose. Compared to baseline, serum glutamate levels rose about 2-fold within 30 min, falling to baseline levels in 1 h. Tung and Tung [23] concluded that infants also have considerable ability to metabolize large oral doses of glutamate.

McLaughlan et al. [24] assessed the toxicokinetics of glutamate at bolus oral doses of 0, 50, 100, 200, 400, 500, 2,000, 4,000, and 8,000 mg MSG/kg bw given to male weaning Wistar rats (90–110 g). McLaughlan et al. [24] reported that plasma concentrations of glutamate were not appreciably increased at a dose of 100 mg/kg bw, with plasma concentrations increasing at higher doses in a dose-proportional manner. At 200 mg/kg bw, oral bolus dosing with MSG along with meat resulted in an approximately 1.7- to 3.5-fold increase in plasma glutamate concentrations 30 min post-dosing. These data are very similar to the results reported in humans using similar bolus doses.

The work of Stegink et al. [15], Tung and Tung [23], and McLaughlan et al. [24] demonstrates that the threshold for systemic exposure to glutamate following oral dosing in both rats and man is in the range of 100–150 mg/kg bw. As a result, at least at these doses, the toxicokinetics of glutamate in both rats and man are equivalent (i.e., no, or similar fold increases, in plasma glutamate concentrations).

Furthermore, glutamate does not freely cross the blood-brain barrier. The concentration of glutamate in the brain (10,000–12,000 μM) is much higher than in plasma (30–100 μM), with the concentration gradient being maintained through glutamate transporters. Glutamate can access the brain through areas not contained within the blood-brain barrier. A number of studies demonstrated that even at high oral doses up to 4 g/kg bw/day, there is little impact on glutamate concentrations in the central nervous system [15, 24–28]. Studies which have assessed exposures of the brain to dosed glutamate have shown that even at a high bolus dose of up to 4,000 mg/kg bw in adult animals [21], no significant changes in brain glutamate levels occurred. Studies where glutamate levels in the brain can be increased through high oral doses, are limited to neonatal animals with less developed blood-brain barriers.

Based on human studies [29, 30], there appears to be little impact of intake of MSG at a dose of 6 g on breast milk glutamate concentrations. Furthermore, intake of glutamate from mother's milk by breast-fed babies, which can be considered of no concern, may amount to levels up to 27–32 mg/kg bw/day, as can be calculated based on the level of free glutamate in breast milk reported to amount to

1,529 $\mu\text{mol/L}$ (equal to 225 mg/L) [31], assuming intake of 600 or 1,000 mL by a 2 or 6 months old infant of 5 or 7 kg bw. Protein hydrolysate infant formula contains much more glutamate than breast milk [32]. A survey of the free amino acid content in protein hydrolysate infant formula revealed the presence of glutamate at concentrations of up to over 8,000 $\mu\text{mol/L}$ (equal to about 1,200 mg/L). Using the same assumptions, this would result in intakes amounting to about 144–171 mg/kg bw/day.

Overall, the available ADME (absorption, distribution, metabolism, and excretion) data indicate that: (a) much of orally consumed glutamate is used as an energy source by intestinal cells and, as a result, plasma levels of glutamate are not significantly impacted by oral intake, except at large bolus doses administered in water; (b) bolus dosages in rodents result in higher plasma levels than in humans; (c) high oral doses of glutamate do not significantly increase brain concentrations; (d) oral doses of glutamate do not appear to impact fetal plasma glutamate levels or concentrations in breast milk, (e) toxicokinetics of glutamate in both rats and man are equivalent showing no, or similar fold increases in plasma glutamate concentrations upon dietary intake, and (f) the dietary mode of administration in the Vorhees et al. [3] study is likely to have resulted in only limited, if any, exposure of the offspring, including the fetal and pre-weaning stages.

Hazard Identification and Assessment

Human Data

Exposure to glutamate has been related to the so-called “MSG complex syndrome” as a de facto condition although previous evaluations conducted by the Federation of American Societies for Experimental Biology (FASEB) [33] concluded that there may exist a subgroup of individuals in the healthy population who may respond to greater than 3 g of MSG under certain conditions. Later, the Food Standards Australia New Zealand [34] concluded that the studies available at that time largely failed to demonstrate a causal relationship between “Chinese Restaurant Syndrome” and consumption of MSG. While noting that symptomology has been experienced by individuals in a clinical setting during the consumption of large doses of MSG in the absence of food, such effects are attenuated by the consumption of food. Since the FASEB evaluation [33], a newer double-blind, multi-centre epidemiology study failed to demonstrate any reproducible responses to 5 g of MSG when administered during the course of a meal [35]. Likewise, the review by Williams and Woessner [36] re-iterated previous conclusions that no evidence of symptoms has been associated with the

Table 1. Overview of human studies on MSG considered adequate by the EFSA [2]

Reference	Study design and result
[39]	5,000 mg MSG in a meal: no evidence for symptoms in humans
[40]	Sensitive subjects (5,000 mg) double blind placebo-controlled double-blind study (0/1,250/2,500/5,000 mg) in citrus beverage: frequency reported symptoms show dose-response and NOAEL at 1,250 mg
[35]	Multicenter, double-blind, placebo-controlled, multiple-challenge study in 5,000 mg sensitive subjects; dose-response (0/1,250/2,500/5,000 mg citrus flavored beverage) and significant response at all dose levels but not reproducible; no response when 5,000 mg MSG dosed in a meal
[36]	Review: data till that time: no symptoms up to 3,000 mg and in a meal 3,000 up to 5,000 mg: symptoms observed especially on an empty stomach

consumption of up to 3 g of MSG per meal. They further state that symptoms have occurred with MSG doses in the 3–5 g (as a single bolus) range, but only in the absence of food on an empty stomach. However, it is important to point out that glutamate is consumed within the diet and not on an empty stomach as a bolus dose. There is no substantive evidence to demonstrate that symptoms associated with “MSG symptom complex” occur to any extent when glutamate is consumed as part of the diet. Hence, concerns about “MSG symptom complex” are largely historical and scientifically unfounded and not supported by empirical evidence, especially in relation to the known compartmentalization of the metabolism of glutamate. As a result, it is considered inappropriate to infer the existence of “MSG symptom complex” as an underlying concern to suggest neurotoxic potential of glutamate.

Human studies are often preferred for the assessment of possible risks from the ingestion of food additives, as outlined by the EFSA [37]. In their opinion on glutamate, the EFSA concluded that 4 human studies are adequate [2]. Table 1 provides an overview of these studies. This overview corroborates the difference when MSG is dosed in a meal or not. Given that the use as a food additive implicitly means use in a food matrix, especially the 3 studies testing MSG in a meal are relevant. These studies point to the absence of adverse effects at dose levels up to at least 5,000 mg in a meal, equal to 70 mg/kg bw/day for a 70 kg person. Given that these studies were performed in glutamate sensitive subjects, using these data to define a health-based guidance value would not require use of an uncertainty value for inter-individual differences. Given the absence of any effect, and especially of a dose-response in these human studies where MSG was dosed in a meal, the EFSA concluded that the studies were not suitable to be used for the derivation of a health-based guidance value. However, these studies do point out that adverse effects in sensitive

individuals are not expected at dose levels of at least 70 mg/kg bw/day, and this value could therefore be defined as an upper safe level, which is defined by the Institute of Medicine (IOM) as the highest level of daily chronic nutrient intake that can be consumed by sensitive members of the general population without expectation of adverse effect [38]. This value is substantially higher than the now established ADI.

Furthermore, levels of free glutamate in breast milk may be as high as 1,529 $\mu\text{mol/L}$ [31], which is equal to 225 mg/L and thus, for a 2–6 months old infant of 5–7 kg bw, drinking 600–1,000 mL mother’s milk per day would result in intakes around or even exceeding the now established ADI. The above considerations already indicate that the ADI of 30 mg/kg bw/day now proposed by EFSA is overprotective.

Animal Data

When adequate human data are unavailable, *in vivo* studies using experimental animals can provide the basis to assess the risks to humans from ingestion of food additives [37]. As already indicated, this is done through identification of a NOAEL or BMDL from a suitable key study, usually a 90-day rodent or long-term carcinogenicity/toxicity study, to which appropriate “safety” or “uncertainty” factors are applied. Table 2 presents an overview of the available 90-day rodent studies, performed according to OECD (Organisation for Economic Cooperation and Development) Test Guideline 408, and the available chronic 2 year dietary studies on MSG.

From this overview, it appears that none of the studies report adverse effects indicating NOAEL values were higher than the highest dose levels tested. The two 90 day studies tested MSG up to a highest dose level of 5% in the diet, resulting in NOAEL values of around 3 g/kg bw/day. The absence of any adverse effect at such high dietary

Table 2. Overview of available 90 day and 2 year dietary studies on MSG in rodents

Study type	Species	Dose levels tested mg/kg bw/day	Effects	NOAEL mg/kg bw/day	Reference
90 days OECD TG 408 study	Rats	0/308/931/3,170 males 0/354/1,066/3,620 females	No test substance related effects	>3,170	[41]
90 days OECD TG 408 study	Rats	0/700/1,300/2,700 males 0/700/1,500/2,900 females	No test substance related effects	>2,700	[42]
2 years study	Mice	0/58/201 males 0/53/183 females	No effects but high mortality in all groups	>183	[43]
2 years study	Rats	0/450/900/1,800 males 0/580/1,160/2,320 females	No effects	>1,800	[44]
2 years study	Rats	0/59/133 males 0/33/73 females	No effects	>73	[45]
2 years study	Rats	0/231/481/975/1,982 males 0/268/553/1,121/2,311 females	No effects	>1,982	[46]

Table 3. Overview of available reproductive and developmental oral toxicity studies on MSG judged adequate by EFSA [2]

Study type	Species	Dose levels tested mg/kg bw/day	Effects	NOAEL mg/kg bw/day	Reference
3-generation reproductive toxicity study	Mice	0/1,500/6,000 males 0/1,800/7,200 females	No effects, also no brain lesions in histopathology	>6,000	[47]
OECD TG 416 2-generation reproductive toxicity	Rats	0/939/3,131 males 0/1,039/3,496 females	No effects	>3,131	[53]
OECD TG 414 Pre-natal developmental toxicity study	Rats	0/302/898/ 3,019 from GD 6–20 pregnancy	No effects	>3,019	[54]
Developmental neurotoxicity study	Rats	0/1,900/3,700/5,300 males, 0/1,600/3,200/5,000 females in the pre-breeding period	High-dose: delayed early swimming development, diminished rearing frequency in open field, altered active avoidance acquisition and extinction, and prolonged day-2 passive avoidance retention	3,200	[3]
Developmental neuro behavior study	Rats	0 and 10,000 GD 7–20 pregnancy	EFSA [2]: no effects when corrected for multiple testing	>10,000	[55, 56]

dose levels of 5% in the diet illustrates the difficulties in defining adequate points of departure for risk assessment for macronutrients from studies in laboratory animals, since this would require even higher dose levels than 5% in the diet which might result in nutritional and metabolic imbalances that would yield secondary adverse effects, including weight loss and increased mortality [1].

The 90-day and 2-year chronic toxicity studies did not indicate potential neurotoxic effects. However, the fact that direct exposure of neonates via subcutaneous or in-

traperitoneal injection of non-physiological high doses of MSG caused discrete brain lesions which, at least in part, might also relate to endocrine disturbances observed in later life [47] (and references therein) triggered reproductive and (neuro)developmental studies via the oral route, but these adverse effects, including the endocrine disturbances, were not found upon dietary administration of MSG even at very high doses [44, 48–52]. Table 3 provides an overview of the available reproductive and (neuro)developmental toxicity studies judged adequate by EFSA.

The pre-natal developmental toxicity study reported by Vorhees et al. [3] was selected by the EFSA to define the point of departure for defining the ADI, mainly because this was the only study reporting adverse effects at the highest dose level tested. The other studies presented in Table 3 did not show effects up to the highest (or only) dose level tested, which were similar or even higher than the NOAEL derived by EFSA from the Vorhees et al. [3] study. Especially, a 3-generation reproductive toxicity study in mice reported by Anantharaman [47] is of interest, because it included detailed histopathology of the brain and did not report any adverse effects up to the highest dose level tested of 6,000 mg/kg bw/day. This study reported by Anantharaman [47] selected mice as the experimental model, because mice were considered the most sensitive species. Repeated subcutaneous or intraperitoneal administration of MSG to new-born mice at dose levels above 0.4 up to 4 or 5 g/kg bw/day resulted in discrete brain lesions, mainly in the pre-optic and arcuate nuclei of the hypothalamus, together with scattered neurons within the median eminence [50, 57, 58]. Bolus oral dosing via an aqueous solution at dose levels of 0.5 up to 2.0 g MSG/kg bw to 10–12 days old mice also resulted in damage of the arcuate nuclei [57], although administration of MSG in the diet at levels up to 42 g/kg bw/day did not result in these symptoms [44, 50, 52]. Anantharaman [47] investigated neuronal densities especially in the arcuate and other nuclei of the hypothalamus, in the basal ganglia, in the hippocampus formation and the thalamus as well as in the cortex. The study also specifically investigated the presence of ganglial cell degeneration and necrosis, phagocytosis of decaying ganglial cells, decreased density in ganglial cells, especially of the hypothalamic nuclei, disturbed bilateral symmetry of ganglial cell pattern, and glial proliferation, altered glial cells or evidence of edema and myelin changes. No effects were observed at dietary dose levels up to 6,000 mg/kg bw/day, the highest dose tested. These observations indicate that in a sensitive animal model for assessing human safety, at higher dose levels than tested in the Vorhees et al. [3] study, no underlying pathology was observed that would support observations related to developmental neurotoxicity. This consideration triggers the need for a careful re-evaluation of the Vorhees et al. [3] study.

Re-Evaluation of Vorhees et al. [3]

The study reported by Vorhees et al. [3] was a preliminary analysis designed to evaluate the potential for a developmental test battery to detect potential neurotoxins and was developed under contract with the United States

Food and Drug Administration that also selected the test articles and doses used in this battery.

While the authors of the study report several changes in the performance in the open field, swimming, and active and passive avoidance tests that are concluded to be treatment-related and considered as adverse in nature by the EFSA Panel [2], there are significant concerns regarding the appropriateness of using this study for deriving an ADI. A recent evaluation of the study by the first author concluded that the findings of the study do not exhibit a pattern of developmental toxicity (Vorhees et al. [3], this issue). Furthermore, this study has been available since 1979 and has not been used for the purpose of safety assessment in any previous regulatory review of glutamate and the study is not mentioned in the latest JECFA monograph on amino acids and related substances [7].

It is also of importance to note that the study was designed to specifically evaluate a potential new test battery for neurodevelopment studies with none of the parameters eventually ending up in current OECD Guidelines for neurodevelopmental toxicity testing [59], because of a high degree of variability, leading eventually to the conclusion that these findings do not exhibit a pattern of developmental neurotoxicity (Vorhees et al. [3], this issue).

Taking this together, and considering all available reproductive and (neuro)developmental toxicity studies (Table 3), it is concluded that the preliminary nature of the Vorhees et al. [3] study calls into question the conclusions drawn by EFSA, and that results from the study of Anantharaman [47] provide a better point of departure for risk assessment of MSG. This study indicates that the point of departure for deriving an ADI from animal data is likely even higher than 6,000 mg/kg bw/day. The developmental neurobehavior study reported by Frieder and Grimm [55, 56] on which the EFSA concluded that there were no adverse effects when correcting for multiple testing, indicates that the NOAEL value for neurodevelopmental effects may be even higher than 10,000 mg/kg bw/day.

Exposure

Exposure to glutamate occurs through consumption in the diet either in the free form or bound in protein as well as through consumption of food products to which glutamate has been added as an additive. It has been reported that a typical Western diet results in total glutamate exposures in the range of 5–12 g/day [60], including anywhere from 0.5 to 5.0 g of free glutamate added to food as an additive. A recent conservative Tier II estimate conducted by TNO [42] found MSG exposure in The Netherlands ranging from 0.9 to 2.5 g/day and exposure to all other free

glutamates up to 2.9 g/day. Free glutamate does not include glutamate derived from protein sources. Compared to the EFSA-derived ADI of 30 mg/kg bw/day [2], or about 2.1 g/day for the default 70 kg adult, as can be seen from these data, this ADI value is in the range of, or even below, the dietary intake of free glutamate excluding foods to which glutamate has been added as an additive.

Potential dietary intakes from protein are likely to be even higher than the 30 mg/kg bw/day ADI derived by the EFSA [2]. Glutamate from protein, once hydrolyzed to the component amino acid, will be handled metabolically exactly in the same manner as free glutamate. Thus, the ADI of 30 mg/kg bw/day is in all likelihood lower than the intake of glutamate from normal dietary consumption. Likewise, the intake analysis performed by the EFSA [2] showed that the 95th percentile estimates ranged to over 30 mg/kg bw/day in all age categories, with exposures of up to 198 mg/kg bw/day in infants.

The intake data indicate that even without added glutamate, for most age categories, consumption of glutamate exceeds the 30 mg/kg bw/day ADI established by the EFSA [2]. This ADI is likely to be well exceeded by populations when consumers of high protein diets are included within exposure analysis. Furthermore, levels of free glutamate in breast milk may be as high as 1,529 $\mu\text{mol/L}$ [31], which is equal to 225 mg/L and thus, for a 2–6 months old infant of 5–7 kg bw, drinking 600–1,000 mL mother's milk per day would result in intakes around or even exceeding the now established ADI. Clearly, there is a disconnect between the results of the EFSA's hazard assessment (i.e., establishment of an ADI of 30 mg/kg bw/day from Vorhees et al. [3]) and the reality that there is no evidence of adverse effects, including potential for neurotoxicity, in any population subgroup at current levels of glutamate intake. This disconnect arises not only due to the selection of the Vorhees et al. [3] study for use in deriving the ADI, but also from the use of a food additive NOAEL/BMDL-safety factor/uncertainty factor risk assessment paradigm to establish an ADI in the first place. It should be noted that the NOAEL in the Vorhees et al. [3] study was $\sim 3,200$ mg/kg bw/day. This dose is closer to the limit of what can be administered to rodents via the diet. Dietary concentrations of 5% have been considered to be the maximum feasible concentrations that can be fed in rodent toxicity studies without risk of secondary effects arising from nutritional or metabolic imbalance [1]. This dietary concentration equates to about 2,500 mg/kg bw/day (i.e., lower than the "NOAEL" from the Vorhees et al. [3] study). Likewise, in oral gavage dosing studies, 2,000 mg/kg bw/day is considered the "limit"

dose in subchronic rodent toxicity studies [61]. As a result, the NOAEL identified by the EFSA [2] from the Vorhees et al. [3] study is in the range of NOAEL values that could be established from any subchronic toxicity study using the highest feasible doses. This fact highlights the problem of using the NOAEL/BMDL-safety/uncertainty factor paradigm for macronutrients.

Use of the Default Approach for Risk Assessment of Macronutrients

The need for an alternative approach to the safety assessment of macro ingredients and particularly macronutrients is not new, as evidenced by publications on the topic over the past 25 or more years [38, 62–65].

In 1992, Dr. Joseph Borzelleca published a seminal article that presented an overview of the problem of safety assessment of macronutrients with potentially large intakes. Borzelleca [62] overtly states that "For macronutrient substitutes, other approaches to establishing the ADI are more appropriate (than the ADI-NOAEL/BMDL-SF/UF approach) because of the high intakes of these materials compared with more traditional food additives." Dying et al. [64] in their discourse on hazard characterization of food and diet, in relation to dose-response and mechanisms of action also noted that macronutrients represent a special class that cannot follow "routine assessment protocol(s)." More recently, Rodricks [38] has discussed the limitations of current approaches to risk assessment of macronutrients, including amino acids. For amino acids in particular, Rodricks [38] noted that the IOM had attempted to establish "Risk-based Upper Limit" values for amino acids to assess potential risk from excessive intakes of amino acids, particularly from dietary supplements. 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. By definition, a UL does not have a pre-determined safety factor (e.g., 100-fold) included in the value.

A New Approach for Risk Assessment of Glutamate

Given the re-evaluation of the animal toxicity data presented above, and the fact that rodents have been shown to be more susceptible to high bolus doses relative to primates and thus may not be an appropriate model from which to assess the potential, if any, for glutamate neurotoxicity in humans, there is a need for a more scientifically robust approach for the risk assessment of glutamate.

First of all the point of departure of 3,200 mg/kg bw/day from the Vorhees et al. [3] study needs to be reconsid-

ered given the fact that effects reported were inconsistent across sexes, not consistent over testing days, did not show dose-response behavior, and/or were not accompanied by any underlying pathology. Furthermore, the endpoints tested were in an experimental stage and were never included in later OECD protocols for neurodevelopmental toxicity testing [59], because of a high degree of variability, leading eventually to the conclusions that these findings do not exhibit a pattern of developmental neurotoxicity (Vorhees et al. [3], this issue). In addition, other reproductive and (neuro)developmental toxicity studies reveal higher NOAEL values, including the highest dose level tested of 6,000 mg/kg bw/day in the 3-generation reproductive toxicity study in mice as the most sensitive species reported by Anantharaman [47] that also performed extensive histopathology of brain tissue, and the only dose tested of 10,000 mg/kg bw/day in the developmental neurobehavior study reported by Frieder and Grimm [55, 56] on which the EFSA concluded that there were no adverse effects when corrected for multiple testing. Considering the study of Vorhees et al. [3] to be inappropriate for human risk assessment, these other studies provide a point of departure that is higher than 6,000 mg/kg bw/day and perhaps even higher than 10,000 mg/kg bw/day.

Furthermore, the available data support reconsideration of the traditional default uncertainty factor of 100 used for food additives when assessing the safety of a macronutrient. In regard to glutamate, one approach would be to still use the food additive risk assessment paradigm but in this case take a more empirical approach to deriving a health-based guidance value through critical assessment of the default uncertainty factors that comprise the traditional 100-fold factor that is usually applied to a NOAEL or BMDL value identified from a toxicology study. The default 100-fold uncertainty factor is composed of two 10-fold factors, to take account for interspecies variation and inter-individual differences. The 10-fold uncertainty factor for interspecies variability can be further subdivided into 2 uncertainty factors: a 4.0-fold factor to account for potential interspecies differences in toxicokinetics and a 2.5-fold factor for uncertainties in toxicodynamic responses [66]. For inter-individual differences (i.e., within the human population), the 10-fold uncertainty factor can also be subdivided into a 3.16-fold factor for each of potential toxicokinetic and toxicodynamic differences [66].

For glutamate, extensive pharmacokinetic and metabolism data are available for both rats and humans. Such data can be used to define a chemical-specific uncertainty factor (CSAF) for either or both of interspecies and intraspecies differences.

As described above, the work of Stegink et al. [15], Tung and Tung [23], and McLaughlan et al. [24] demonstrates that following oral bolus dosing in the presence of other dietary ingredients in both rats and man in the range of 100–150 mg/kg bw, the toxicokinetics of glutamate in both rats and man are similar (i.e., no, or similar fold increases, in plasma glutamate concentrations). As a result, the default uncertainty factor for interspecies differences in toxicokinetics can be reduced at least from 4 to 1.

While a wealth of additional toxicokinetic and metabolic data are available in both rats and humans, as well as in other species, it is difficult to quantify a CSAF for toxicodynamics (both inter- and intraspecies) or for intraspecies toxicokinetic differences (i.e., inter-individual variation in humans). This is due to the fact that much of the data in rats result from high- and/or bolus-dose studies (i.e., upwards of 8 g/kg bw/day) for which corresponding data in man do not exist. Despite this, the default uncertainty factors for the remaining endpoints are considered especially conservative since the available data do show that the metabolism in both rats and man is similar. In addition, there are no indications of significant interspecies or intraspecies differences in the physiological role of glutamate or in its toxicodynamic properties. In fact, the available data indicate that neonatal rodents are likely the most sensitive species in regard to manifestation of toxic properties (i.e., neurological lesions) of excess exposure to glutamate [34].

Therefore, based on the above CSAF approach, the uncertainty factor for interspecies differences when calculating an ADI for glutamate can be revised downward to at least 2.5 (i.e., 1 for toxicokinetic $\times$ 2.5 [default value] for toxicodynamic differences). The uncertainty factor for intraspecies differences would remain at the default value 10 (i.e., 3.16 for toxicokinetic $\times$ 3.16 for toxicodynamic differences). As a result, the default 100-fold uncertainty factor would be reduced to 25.

The use of this CSAF of 25 together with the point of departure from the 3-generation reproductive toxicity study reported by Anantharaman [47] of at least 6,000 mg/kg bw/day, being the highest dose tested, would result in an ADI of at least 240 mg/kg bw/day.

Comparison of this ADI to the dietary exposure to glutamate from its use as food additive (E620–E625), as calculated by EFSA in their refined non-brand loyal exposure scenario, reveals that exposure at both the mean and at the 95th percentile remains far below this value of at least 240 mg/kg bw/day.

Discussion and Conclusion

The EFSA re-evaluation of glutamate [2] marks a significant departure from past authoritative reviews as the EFSA selected a neurodevelopmental toxicity study, performed in rats, [3] to establish a group ADI of 30 mg/kg bw/day, whereas previous reviews by JECFA and others came to the conclusion that this macronutrient poses no safety concerns at levels of current dietary consumption. The study selected from where to derive the new group ADI, was available and assessed in previous reviews. The results of the Vorhees et al. [3] study have not been previously used in the hazard characterization of glutamate. In addition, the latest EFSA evaluation [2] highlighted the pitfalls in applying the traditional NOAEL/BMDL-safety/uncertainty factor approach to the risk assessment of macronutrients. The use of the Vorhees et al. [3] study only compounds this issue.

The Vorhees et al. [3] study is not appropriate for use in hazard characterization as its protocol was designed as part of a program to develop protocols for the conduct of neurobehavioral/neurodevelopmental toxicity tests. At the time of its conduct, MSG was likely selected as a test candidate on the basis of the neonatal rodent studies (pups having less intact blood-brain barrier function), which showed neurotoxic potential of MSG, but only at high bolus doses and only during the early neonatal period in sensitive rodent species. These studies are now considered to be of no relevance to the safety assessment of MSG or other glutamates as present in the diet.

The results of the Vorhees et al. [3] study are difficult to interpret in light of what is known about the absorption and metabolism of glutamate included as part of the diet. Exposures to glutamate during gestation, pre-weaning, and post-weaning were not quantified, but glutamate exposures were likely low during these periods due to (1) maternal metabolism in the gut and liver (gestation and pre-weaning), (2) limited excretion in the dam's milk (pre-weaning), and (3) insufficient exposures to achieve any significant change in brain glutamate concentrations (late pre-weaning and post-weaning phases). Even if effects are associated with decreased blood-brain barrier function during gestation and/or the early neonatal periods, they are of no relevance to humans given the exposures of neonates to glutamate via breast milk at levels equivalent to or higher than the derived ADI of 30 mg/kg bw/day. Furthermore, exposure of infants to glutamate from consumption of extensively hydrolyzed protein infant formula exceeds that from breast milk. Infant exposure to glutamate has been estimated to range from a mean of 198 to 489 mg/kg bw/

day at the 95th percentile [2], with the upper range likely the result of the consumption of protein hydrolysate infant formulae. The fact that infants of 2–6 months of age, a sensitive period in the development of the blood-brain barrier, consume levels of free glutamate in breast milk at intakes around or even exceeding the EFSA derived ADI, questions the applicability of glutamate having neurobehavioral toxicity [31]. Furthermore, the endpoints tested were never included in later OECD protocols for neurodevelopmental toxicity testing [59], because of a high degree of variability, leading eventually to the conclusions that these findings do not exhibit a pattern of developmental neurotoxicity (Vorhees et al. [3], this issue).

Given the above, it is considered inappropriate to establish an ADI of 30 mg/kg bw/day from the Vorhees et al. [3] study, also in light of the fact that this ADI is within the range of free glutamate consumption from food to which additional glutamate has not been added, and in the range of intake for breast-fed babies. Glutamate exposure from normal protein consumption also exceeds the ADI of 30 mg/kg bw/day. This unrealistic situation is the result of the inappropriate application of the food additive risk assessment paradigm and the use of a 100-fold uncertainty factor to the NOAEL identified by EFSA [2] based on the Vorhees et al. [3] study.

Caution should be exercised when applying uncertainty factors to NOAEL values obtained from laboratory animal toxicity studies that were developed for the risk assessment of food additives consumed at low levels for macronutrients. Other alternative approaches need to be considered for food ingredients with high levels of intake and which cannot be satisfactorily studied in laboratory animals at supraphysiological doses (i.e., >5–10% in the diet) [38, 62, 63, 65, 67]. All such approaches engender the dropping of strict adherence to a safety margin approach and entail the review of available data in a more holistic manner, whereby a safety threshold is established largely on the basis of human data and through critical analysis of the ingredient's mechanism(s) of action, role within the body, and means of metabolism and elimination.

The data available for amino acids such as glutamate have previously been considered unsuitable for application in alternate approaches for deriving a safety-based upper limit of exposure (i.e., UL) [38]. However, even when employing this classical risk analysis paradigm, the extensive toxicokinetic and metabolism data available for glutamate can be considered for use in deriving a CSAF that can replace the default 100-fold uncertainty factor. Based on toxicokinetic data, the uncertainty factor for interspecies differences between rat and man can be re-

duced from 10 to 2.5 given that the toxicokinetics of glutamate, when given as a bolus dose or administered as part of the diet, is similar in both species. This CSAF reduces the default 100-fold uncertainty factor to at least 25.

In addition, other reproductive and (neuro)developmental toxicity studies reveal higher NOAEL values than the value of 3,200 derived by the EFSA [2] from the Vorhees et al. [3] study. This includes the highest dose level tested of 6,000 mg/kg bw/day in the 3-generation reproductive toxicity study reported by Anantharaman [47] that also performed extensive histopathology of brain tissue, and the only dose tested of 10,000 mg/kg bw/day in the developmental neurobehavior study reported by Frieder and Grimm [55, 56] on which the EFSA concluded that there were no adverse effects when corrected for multiple testing. Considering the study of Vorhees et al. [3] to be inappropriate for human risk assessment, these other studies provide a point of departure that is higher than 6,000 mg/kg bw/day and perhaps even higher than 10,000 mg/kg bw/day.

Furthermore, there is no evidence that current intakes, or even elevated intakes, of glutamate have posed any safety concerns while at the current state of the art it is agreed that concerns about “MSG complex syndrome” are largely historical and unfounded and not supported by empirical evidence, especially in relation to the known compartmentalization of the metabolism of glutamate. As a result, it is not appropriate to infer the existence of “MSG complex syndrome” to suggest the neurotoxic potential of glutamate.

Altogether the group ADI of 30 mg/kg bw/day recently proposed by the EFSA [2] is called into question on the basis of the current evaluation of toxicokinetic data and the Vorhees et al. [3] study, as well as the appropriateness of using a classical food additive risk assessment paradigm for a macronutrient. Use of a CSAF of 25 together with the point of departure from the 3-generation reproductive toxicity study reported by Anantharaman [47] of

at least 6,000 mg/kg bw/day, being the highest dose tested, results in an ADI of at least 240 mg/kg bw/day. In fact, given that (i) 6,000 mg/kg bw was the highest dose level tested in the Anantharaman [47] study and that a dose of 10,000 mg/kg bw/day in a developmental neurobehavior study reported by Frieder and Grimm [55, 56] for which the EFSA concluded that there were no adverse effects, and that (ii) the current estimated dietary exposure to glutamate (E620–E625) resulting from their consumption in the diet and as food additives at both the mean and at the 95th percentile remains far below this value of at least 240 mg/kg bw/day [2], there is no compelling evidence to indicate that the previous ADI of “not specified” warrants any change.

Ethics Statement

The authors have no ethical conflicts to disclose.

Disclosure Statement

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Author Contributions

All authors contributed equally to writing the manuscript.

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