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A chemical-specific adjustment factor for human interindividual differences in kinetics for glutamates (E620-625)

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ABSTRACT

Use of a default methodology for establishment of a health-based guidance value (HBGV) resulted in a group acceptable daily intake (ADI) for glutamates (E620-625) below the normal dietary glutamate intake, and also lower than the intake of free glutamate by breast fed babies. Use of a chemical-specific adjustment factor (CSAF) may overcome this problem. The present study investigates the interindividual human variability in glutamate plasma and brain levels in order to define a CSAF for the interindividual variation in kinetics, a HK_{AF}, for glutamates. Human clinical data on plasma glutamate levels available from different groups of subjects at Mitsui Memorial Hospital as well as literature data on plasma and brain-related glutamate levels were collected and analysed. The median HK_{AF} value obtained amounted to 2.62–2.74 to 2.33–2.52 for plasma derived values and to 1.68–1.81 for brain derived values. Combining these values with the CSAF for the interspecies differences in kinetics of 1 and the default factors for interspecies and interindividual differences in dynamics of 2.5 and 3.16 results in an overall CSAF of 16–20. Using this CSAF will result in a HBGV for glutamate that is no longer below the acceptable range of oral intake (AROI).

1. Introduction

Glutamate is a non-essential amino acid that is authorised for use as a food additive (flavour enhancer) in the form of glutamic acid and its sodium, potassium, calcium, ammonium and magnesium salts (E620-625). In 2017 the European Food Safety Authority (EFSA) adopted an opinion in which they proposed a group acceptable daily intake (ADI) for glutamate and its salts (referred to as glutamate hereafter) of 30 mg/kg body weight (bw)/day (EFSA, 2017). Besides its addition as a food additive, glutamate in its free form is also naturally present in a wide variety of foods. Mean dietary intake of free glutamate from naturally occurring sources in European adults was recently estimated to be 8–17 mg/kg bw/day with high consumers consuming up to 11–36 mg/kg bw/day. Glutamate intake from food additive sources was estimated to be 2–13 mg/kg bw/day (mean) and 7–35 mg/kg bw/day (high consumers) (Tennant, 2018). In a further evaluation of this ADI the value appeared to be below the normal dietary glutamate intake, and also lower than the intake of free glutamate by breast fed babies or by babies fed protein hydrolysate infant formula (Roberts et al., 2018). This illustrates that for glutamate and other (macro)nutrients applying a default uncertainty factor of 100 to a point of departure like a NOAEL (no-observed adverse effect level) or BMDL (lower confidence limit of the benchmark dose) to establish a health-based guidance value (HBGV) may not be appropriate. This conclusion is in line with a WHO report on principles and methods for the assessment of risk from essential trace elements (WHO, 2002), the report of the Joint FAO/WHO Technical Workshop on Food Nutrient Risk Assessment (WHO, 2006), and the recent draft statement of EFSA on the derivation of HBGVs for regulated products that are also nutrients (EFSA, 2020). The EFSA statement indicates that, when defining a HBGV for nutrients, one should keep in mind the concept of an acceptable range of oral intake (AROI) to ascertain establishment of an HBGV within the boundaries of risks related to deficiency or toxicity. EFSA in their draft statement (EFSA, 2020) referred to the EFSA re-evaluation of phosphates as an example (EFSA, 2019). In this re-evaluation of phosphates EFSA applied a

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List of abbreviations

ADI	acceptable daily intake
AD_{AF}	CSAF interspecies differences dynamics
AD_{UF}	default uncertainty factor interspecies differences dynamics
AK_{AF}	CSAF interspecies differences kinetics
AK_{UF}	default uncertainty factor interspecies differences kinetics
AROI	acceptable range of oral intake
BMDL	lower confidence limit of the benchmark dose
bw	body weight
CI	confidence interval
CSAF	chemical-specific adjustment factor
DBP	diastolic blood pressure
EFSA	European Food Safety Authority
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
GM	geometric mean
HbA1c	serum level of hemoglobin A1c
HBGV	health-based guidance value
HD_{AF}	CSAF interindividual differences dynamics
HD_{UF}	default uncertainty factor interindividual differences dynamics
HK_{AF}	CSAF interindividual differences kinetics
HK_{UF}	default uncertainty factor interindividual differences kinetics
HDL-C	serum level of high density lipid cholesterol
IGTC	International Glutamate Technical Committee
IPCS	International Program on Chemical Safety
NOAEL	no observed adverse effect level
SBP	systolic blood pressure
SD	standard deviation
SE	standard error of the mean
TG	serum level of total triglycerides
TTC	threshold of toxicological concern
UF	uncertainty factor

so-called chemical-specific adjustment factor (CSAF) of 4 instead of the default value of 100 to take the interspecies and interindividual differences in kinetics and dynamics into account and to convert the point of departure derived from the toxicity data into a HBGV (EFSA, 2019; Smeraldi et al., 2020). The use of a CSAF becomes feasible when chemical specific data on interspecies and/or human interindividual differences in kinetics and/or dynamics are available.

When defining a CSAF it is taken into account that the 100-fold uncertainty factor for interspecies and interindividual variability can be subdivided into four uncertainty factors (Fig. 1) including a default uncertainty factor 4.0 to account for interspecies differences in kinetics (AK_{UF}), a default uncertainty factor 2.5 for interspecies differences in dynamics (AD_{UF}) and a default uncertainty factor 3.16 for each of potential kinetic and dynamic interindividual differences (HK_{UF} and HD_{UF}) (IPCS, 2005) (Fig. 1). In case chemical-specific adjustment factors are defined the respective symbols get a subscript AF instead of UF and were denoted by the International Program on Chemical Safety (IPCS) as AK_{AF}, AD_{AF}, HK_{AF} and HD_{AF} (IPCS, 2005).

The CSAF of 4 in the EFSA re-evaluation of phosphates resulted from two times a factor 2 for both the interspecies and interindividual uncertainty factor for kinetics, while both values for differences in dynamics were set to 1 because of the similarity in the mode of action and the accompanying histopathology in both rat and human (EFSA, 2019). Enabling such an approach for glutamate requires the definition of a CSAF for glutamate.

Previously it has been argued that the available data on glutamate support reconsideration of the traditional default uncertainty factor of 100 when considering establishment of a HBGV for glutamate (Roberts et al., 2018). This suggestion was based on the extensive pharmacokinetic data available for glutamate in both rats and humans, showing that the kinetics of glutamate in rat and man are similar and thus supporting replacement of the AK_{UF} of 4.0 by an AK_{AF} of 1 resulting in an overall CSAF of 25 instead of 100 to be used when defining a HBGV for glutamate (Roberts et al., 2018). The aim of the present study was to investigate the interindividual human variability in glutamate plasma and brain levels in order to define a CSAF for the interindividual variation in kinetics, a HK_{AF}, for glutamates. To this end, human clinical data on plasma glutamate levels available from different groups of subjects at Mitsui Memorial Hospital as well as literature data on human plasma and brain-related glutamate levels were collected and analysed. In line with the guidelines of the IPCS, the HK_{AF} was calculated as the ratio between a given percentile (95th, 97.5th or 99th) and the geometric mean (GM) of the relevant data (IPCS, 2005).

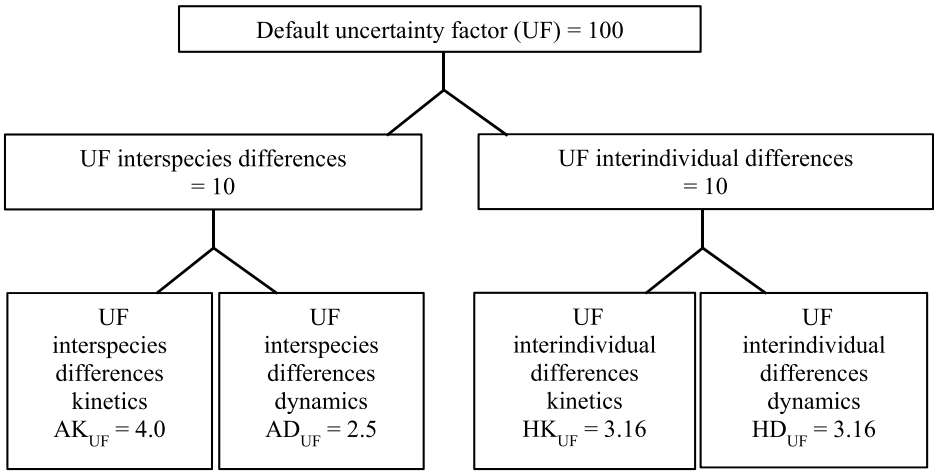

Fig. 1. The subdivision of the default uncertainty factor (UF) 100 into an UF of 10 for interspecies and interindividual differences and further division of these UFs into UFs for kinetic and dynamic differences between species and within the human population, as proposed by WHO/IPCS (IPCS 2005). In case chemical specific adjustment factors are defined the respective symbols get a subscript AF instead of UF and were denoted by IPCS as AK_{AF}, AD_{AF}, HK_{AF} and HD_{AF} (IPCS 2005).

2. Materials and methods

2.1. Ethics

The study at Mitsui Memorial Hospital was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committees of Mitsui Memorial Hospital. All subjects gave their informed consent for inclusion before they participated in the study. All data were analysed anonymously throughout the study. Mitsui Memorial Hospital is the Center for Multiphasic Health Testing and Services.

2.2. Subjects

The main inclusion criteria were as follows; Japanese subjects who had undergone the Ningen Dock comprehensive medical check-up system (Ikeda et al., 2011) in 2018 at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital in Tokyo, not taking antidiabetic medications regularly, not having serious health problems, and at least 20 years old (N = 1000). Patients with hepatitis C or hepatitis B were excluded.

2.3. Analysis of biochemical variables and quantification of glutamate

Blood samples were taken from the individuals after an overnight fast. Fasting plasma glucose (FPG), and serum levels of high density lipid cholesterol (HDL-C), total triglycerides (TG), and hemoglobin A1c (HbA1c) were determined. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. Liver fat content was examined by the ultrasound hepatic/renal ratio. The measurements of other variables were performed as previously described (Yamakado et al., 2012, 2015; Yamamoto et al., 2016).

For the glutamate analyses, blood samples (5 mL) were collected from forearm veins after overnight fasting into tubes containing disodium ethylenediaminetetraacetate and were immediately placed on ice. The plasma glutamate concentrations were measured by high-performance liquid chromatography–electrospray ionization mass spectrometry followed by precolumn derivatization as previously described (Shimbo et al., 2009, 2010; Takehana et al., 2016; Yoshida et al., 2015).

2.4. Literature search

Web of Sciences/Medline/PubMed and Scopus databases were searched up to June 24, 2020 for clinical trials that investigated plasma or brain-related glutamate levels in human. Searches were conducted using the following terms: glutamate; human; plasma (or brain) levels and cross sectional. Included studies provided any of the following parameters of plasma or brain-related glutamate to enable calculation of the HK_{AF}: (1) mean and its standard deviation (SD) or 95% confidence interval (CI), (2) geometric mean (GM) and its SD or 95%CI, and/or (3) graphical data from which quantitative values could be derived. Studies on subjects under medical supervision were excluded which is in line with the IPCS CSAF guidance (IPCS, 2005). Also excluded were studies on dose response models, studies that assessed an outcome other than plasma or brain-related glutamate levels, news briefs, letters, comments, editorials and case reports and presentations.

2.5. Definition of the HK_{AF}

The collected data on glutamate levels included i) individual data sets for plasma glutamate levels that defined distributions, or ii) sets of mean values for human plasma or brain-related levels of glutamate and their SD, or iii) sets of geometric means for human plasma or brain-related levels of glutamate and their SD. The last 2 types of data were used as input for a Monte Carlo simulation using a normal distribution when using a mean value and a log-normal distribution when using a geometric mean value. For the glutamate data of Mitsui Memorial Hospital, a normal distribution of the data was applied based on the nature of the distribution. Also for all literature data normal distribution appeared to adequately describe and match the data, except for the data of populations described by Schmidt et al. (2016). The glutamate data of populations selected from Schmidt et al. (2016), were modelled using a lognormal distribution because the paper indicated that the distribution was logarithmically transformed to approximate the normal distribution. In cases where instead of an SD standard errors (SE) and sample size were provided, the SD was derived from the SE by multiplying the value of SE by the square root of the sample size. In other cases confidence intervals for means were used to calculate SD values. If the sample size is approximately 100 in the respective group, the 95%CI is 3.92 standard errors wide ($3.92 = 2 \times 1.96$). Thus, the SD for the respective group was obtained by dividing the length of the confidence interval by 3.92, and then multiplying by the square root of the sample size.

$$SD = \sqrt{N} \times (upper\ limit - lower\ limit) \Big/ 3.92$$

Model predictions with Monte Carlo simulations were performed with R Statistical Software (Foundation for Statistical Computing, Vienna, Austria). The population distribution generated with the Monte Carlo simulation was statistically analysed with R Statistical Software to calculate the geometric mean, and different percentiles of the plasma or brain-related glutamate values obtained from the Monte Carlo analysis. The population distribution enabled the prediction of the HK_{AF}, which was obtained by dividing the percentile (95th, 97.5th and 99th of the population) of the glutamate levels by the corresponding geometric mean (GM) of the respective distribution. Use of the GM value to define the central tendency value was based on the recommendation in the IPCS guideline to estimate the central tendency of the selected parameter as the simple geometric (or arithmetic, if transformed properly) mean of the relevant data (IPCS, 2005). Thus, the HK_{AF} was calculated as the ratio between the 95th, 97.5th or 99th percentile and the GM of the distribution for the plasma or brain-related glutamate levels for the respective study population subgroup (IPCS, 2005). HK_{AF} values based on all three percentiles are presented since selection of the 95th, 97.5th or 99th percentile as basis for the HK_{AF}, and thus of the actual overall CSAF, is a choice to be made by the risk assessor or risk manager on a case-by-case basis (IPCS, 2005).

From the HK_{AF} values thus obtained one representative value was derived by calculating the median value. Because the median is a representative value for nonparametric analysis and statistically valid to show together with the percentile value of ratio values like the HK_{AF}. It is generally accepted that the mean of a ratio value like the HK_{AF} is not calculated as the mean because each ratio value has a different numerator and denominator which hampers comparability. The histograms of the various distributions were generated with GraphPad (GraphPad Prism 5.0 software, San Diego, CA, USA).

3. Results

3.1. Glutamate plasma levels for different population subgroups at Mitsui Memorial Hospital

Fig. 2 presents the distribution of glutamate plasma levels in different subgroups of subjects at Mitsui Memorial Hospital for which glutamate plasma levels were obtained. This included 131 subjects with metabolic syndrome (Fig. 2A), 49 subjects with diabetes (Fig. 2B), 192 subjects with hypertension (Fig. 2C), 248 subjects with fatty liver (Fig 2D) and 526 healthy subjects (Fig. 2E). Fig. 2F presents the plasma glutamate distribution for all subjects, 1000 in total, taken together. The fact that the number of individual subjects in the different subgroups add up to a higher number of subjects (1146 instead of 1000) is due to the fact that some subjects belong to more than one subgroup.

The distribution for the subjects with metabolic syndrome and

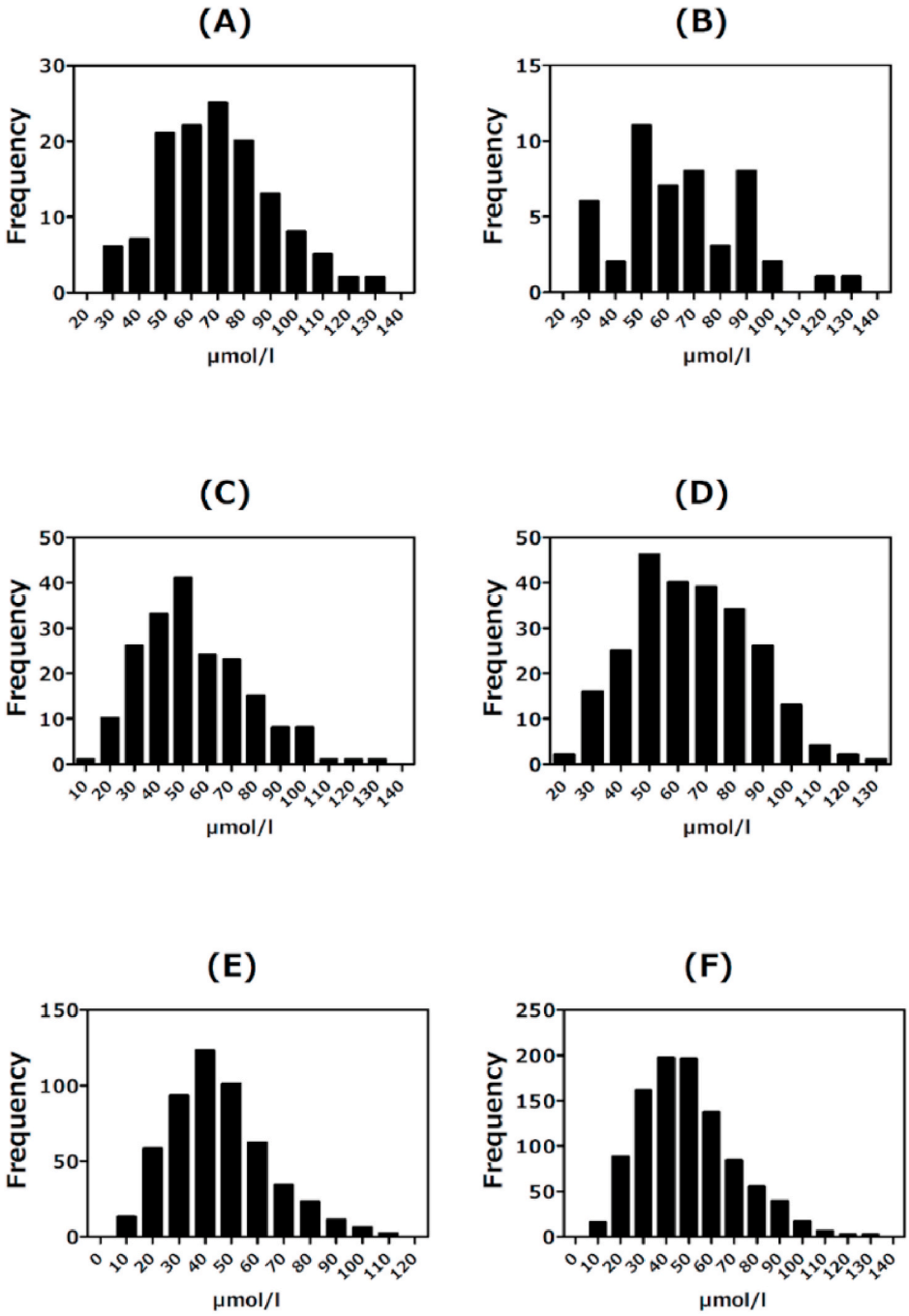

Fig. 2. Distribution of plasma glutamate levels in subgroups of subjects at Mitsui Memorial Hospital including A) 131 subjects with metabolic syndrome, B) 49 subjects with diabetes, C) 192 subjects with hypertension, D), 248 subjects with fatty liver, E) 526 healthy subjects and F) 1000 subjects representing the total population.

Table 1
The GM and 95th, 97.5th and 99th percentile of the distributions presented in Fig. 2 for the plasma glutamate levels of the various subpopulations at Mitsui Memorial Hospital and the group as a whole, and the HK_{AF} values derived from these data.

Population	No of subjects	Plasma glutamate level (µmol/L)				HK_{AF}[a]		
		GM	95th	97.5th	99th	95th	97.5th	99th
Subjects with metabolic syndrome	131	66.55	107.67	120.68	130.15	1.62	1.81	1.96
Subjects with diabetes	49	61.12	101.68	113.32	124.29	1.66	1.85	2.03
Subjects with hypertension	192	49.73	96.24	104.11	123.59	1.94	2.09	2.49
Subjects with fatty liver	248	61.41	101.11	107.51	119.26	1.65	1.75	1.94
Healthy subjects	526	41.07	80.02	90.00	99.56	1.95	2.19	2.42
Total population	1000	44.79	88.25	96.76	106.78	1.97	2.16	2.38

[a] HK_{AF} is calculated as the glutamate plasma level at a given percentile of the distribution divided by the GM (IPCS, 2005).

diabetes (Figs. 2A and B) show normal distributions whereas the other distributions are somewhat right (positively) skewed.

3.2. HK_{AF} values derived from the glutamate plasma level distributions for different subgroups at Mitsui Memorial Hospital

Table 1 presents the geometric mean (GM) and the 95th, 97.5th and 99th percentile of the distribution for the plasma glutamate levels for the different subgroups and also for the population as a whole as presented in Fig. 2. From these values the respective HK_{AF} values for the interindividual differences in glutamate kinetics were calculated as the ratio between the percentiles and the GM (IPCS, 2005). These HK_{AF} values are also presented in Table 1. For the different subpopulations the HK_{AF} varies from 1.62 to 1.95, from 1.75 to 2.19 and from 1.94 to 2.49 using the 95th, 97.5th and 99th percentile, respectively. For the combined population these values amount to respectively 1.97, 2.16 and 2.38, values that are similar to the values of 1.95, 2.19 and 2.47 for the healthy subgroup. From this is follows that the HK_{AF} is lower than the default HK_{UF} of 3.16.

3.3. HK_{AF} values based on distributions for plasma glutamate levels as reported in literature

The literature search revealed several studies reporting data on plasma glutamate levels for subgroups of healthy individuals of different age groups including children and/or subjects on specific diets. Table 2 presents an overview of the respective studies and their populations. Table 2 also presents the GM and 95th, 97.5th and 99th percentile derived from the distributions as presented or derived from the literature data, as well as the HK_{AF} values calculated based on these values. From the results obtained it follows that plasma levels reported appear to vary substantially between studies but not within studies. This can be ascribed to methodological variations and implies that comparisons are only valid within studies. However, given that the HK_{AF} values for interindividual variability are determined as the ratio of values obtained within a study and subgroup, these methodological differences do not affect the HK_{AF} values obtained. For comparison Table 2 also includes the data as obtained for the subpopulations at Mitsui Memorial Hospital.

The HK_{AF} values obtained amount to values from 1.29 to 1.95 at the 95th percentile, from 1.33 to 2.19 at the 97.5th percentile and from 1.34 to 2.49 at the 99th percentile. All values are lower than the default of 3.16. Taking all data together the median of HK_{AF} values amount to 1.64 at the 95th percentile, 1.75 at the 97.5th percentile and 1.82 at the 99th percentile. These values are all substantially lower than the default HK_{UF} of 3.16.

3.4. HK_{AF} values based on Monte Carlo simulations to define the plasma glutamate distributions

Given the limited no of subjects for most of the different populations for which plasma glutamate distribution data were available from literature (Table 2), these data were also analysed using a Monte Carlo simulation in order to increase the number of subjects in the distributions. This Monte Carlo simulation also allows analysis based on the distributions derived from literature data reporting no individual data but only the mean values for human plasma levels of glutamate and their SD or geometric means for human plasma levels of glutamate and their SD. The distributions obtained via this Monte Carlo simulation are presented in Fig. 3. Table 3 presents the GM and 95th, 97.5th and 99th percentile derived from these distributions as obtained by Monte Carlo simulations for 1000 individuals, as well as the HK_{AF} values calculated based on these values. Using the Monte Carlo based analysis (Table 3) allowed inclusion of more literature data sets than when using only the studies with reported individual data (Table 2). This is due to the fact that the Monte Carlo approach only required (geometric) mean and SD values for the glutamate plasma levels to define a distribution for the population of 1000 individuals. For data sets where both analyses were feasible (Tables 2 and 3) the distributions obtained using the Monte Carlo modelling (Table 3) showed somewhat wider distributions resulting in slightly higher HK_{AF} values than derived from the individual data reported in Table 2. This indicates the Monte Carlo approach to be more conservative. The HK_{AF} values obtained via the Monte Carlo simulations vary from 1.04 to 2.49 at the 95th percentile, from 1.05 to 2.71 at the 97.5th percentile and from 1.06 to 2.92 at the 99th percentile. The median of HK_{AF} values amounted to 1.75 at the 95th percentile, 1.85 at the 97.5th percentile and 1.94 at the 99th percentile. All HK_{AF} values were lower than the default of 3.16.

3.5. HK_{AF} values based on Monte Carlo simulations to define brain-related glutamate distributions

Given that brain tissue may be considered the ultimate target organ for glutamate related effects, plasma glutamate levels may be considered a surrogate biomarker. Therefore, an additional effort was undertaken to define HK_{AF} values based on reported brain-related glutamate levels. In this case Monte Carlo simulations based on literature reported mean values for human brain-related levels of glutamate and their SD or geometric means for human brain-related levels of glutamate and their SD were performed. In the studies analysed CerebroSpinal Fluid (CSF) was obtained by lumbar puncture (Perry et al., 1975), striatum data were obtained by scanning the subjects with proton magnetic resonance spectroscopy (1H-MRS) (Caravaggio et al., 2018), hippocampus data were obtained by scanning the subjects with 3-T proton magnetic

Table 2
Overview of data on human plasma glutamate levels in groups of individuals including data from literature, the results of the analysis of the reported data to define the GM and 95th, 97.5th and 99th percentile of the distributions, and the HK_{AF} values derived from these data.

Reference	Population	No of subjects	Plasma glutamate level (μmol/L)				HK_{AF}[a]		
			GM	95th	97.5th	99th	95th	97.5th	99th
Present study	Subjects with metabolic syndrome	131	66.55	107.67	120.68	130.15	1.62	1.81	1.96
	Subjects with diabetes	49	61.12	101.68	113.32	124.29	1.66	1.85	2.03
	Subjects with hypertension	192	49.73	96.24	104.11	231.59	1.94	2.09	2.49
	Subjects with fatty liver	248	61.41	101.11	107.51	119.26	1.65	1.75	1.94
	Healthy subjects	526	41.07	80.02	90.00	99.56	1.95	2.19	2.42
Shimmura et al. (2011)	Healthy male control children	22	20.11	25.99	27.43	28.38	1.29	1.36	1.41
	Male children with high functioning autism (HFA)	23	26.60	44.60	47.80	49.12	1.68	1.80	1.85
Makhro et al. (2016)	Healthy untrained volunteers before exercise	8	37.65	49.98	50.24	50.40	1.33	1.33	1.34
	Healthy untrained volunteers after exercise	8	50.99	76.40	79.20	80.88	1.50	1.55	1.59
	Endurance athletes before exercise	11	33.34	55.50	58.00	59.50	1.66	1.74	1.78
	Endurance athletes after exercise	11	42.39	56.50	57.25	57.70	1.33	1.35	1.36
Droge et al. (1988)	Apparently healthy persons	31	54.39	77.00	81.00	86.40	1.42	1.49	1.59
Median							1.64	1.75	1.82

[a] HK_{AF} is calculated as the glutamate plasma level at a given percentile of the distribution divided by the GM (IPCS 2005).

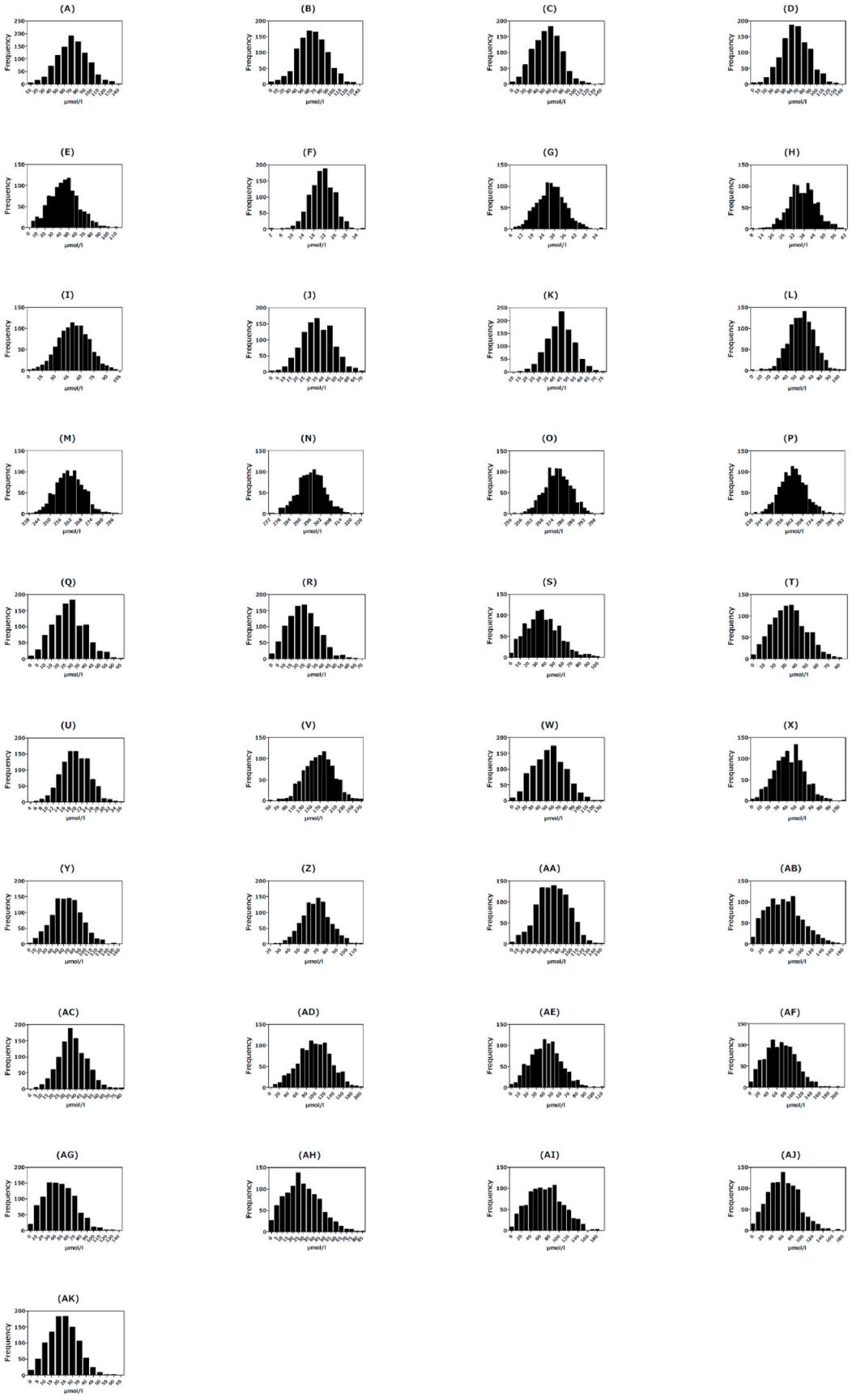

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Fig. 3. Monte Carlo simulation based distributions of plasma glutamate levels in subgroups (N = 1000 in each group) of subjects including.
A) Subjects with metabolic syndrome (Present study), B) Subjects with diabetes (Present study), C) Subjects with hypertension (Present study), D) Subjects with fatty liver (Present study), E) Healthy subjects (Present study), F) healthy male control children (Shimmura et al., 2011), G) male children with high functioning autism (HFA) (Shimmura et al., 2011), H) healthy untrained volunteers before exercise (Makhro et al., 2016), I) healthy untrained volunteers after exercise (Makhro et al., 2016), J) endurance athletes before exercise (Makhro et al., 2016), K) endurance athletes after exercise (Makhro et al., 2016), L) apparently healthy persons (Droge et al., 1988), M) meat-eaters (Schmidt et al., 2016), N) fish-eaters (Schmidt et al., 2016), O) vegetarians (Schmidt et al., 2016), P) vegans (Schmidt et al., 2016), Q) Japanese (Nakamura et al., 2015), R) Korean (Nakamura et al., 2015), S) Chinese (Nakamura et al., 2015), T) athletes (Kamada et al., 2016), U) non-epileptic controls (Janjua et al., 1992), V) healthy controls (Reis et al., 2006), W) control [non-pregnant] woman (Ortega et al., 2003), X) pregnant woman gestational age: ≤ 32 weeks (Ortega et al., 2003), Y) pregnant woman gestational age: > 32 weeks (Ortega et al., 2003), Z) gestational diabetes mellitus (GDM) [fasting] antepartum (Butte et al., 1999), AA) GDM [fasting] Postpartum (Butte et al., 1999), AB) control [fasting] antepartum (Butte et al., 1999), AC) control [fasting] postpartum (Butte et al., 1999), AD) term group (Posod et al., 2017), AE) preterm group (Posod et al., 2017), AF) age group: 0–24 months (Chuang et al., 2002), AG) age group: 2–18 years (Chuang et al., 2002), AH) age group: 19–68 years (Chuang et al., 2002), AI) age group: 0–3 months (Chuang et al., 2002), AJ) age group: 4–24 months (Chuang et al., 2002), and AK) healthy persons (Perry et al., 1975).

resonance spectroscopy (Bossong et al., 2019), and anterior cingulate cortex data were obtained by scanning the subjects with a 3-T whole body magnetic resonance scanner (Hoerst et al., 2010). The distributions obtained via these Monte Carlo simulations are presented in Fig. 4. Table 4 presents the GM and 95th, 97.5th and 99th percentiles derived from these distributions for 1000 individuals, as well as the HK_{AF} values calculated based on these values. The data sets relate to different brain regions but show consistent results. The HK_{AF} values obtained vary from 1.16 to 1.85 at the 95th percentile, from 1.19 to 1.97 at the 97.5th percentile and from 1.22 to 2.15 at the 99th percentile. The median of HK_{AF} values amounted to 1.26 at the 95th percentile, 1.30 at the 97.5th percentile and 1.37 at the 99th percentile. All HK_{AF} values were lower than the default of 3.16 with the median values being up to 1.4 fold lower than the median values obtained based on the plasma distributions of glutamate (Tables 2 and 3).

3.6. *Taking into account potential sensitive subgroups within the population*

Given that the data from the study performed at Mitsui Memorial Hospital relate to different subgroups within the population that were analysed within the same study, they enable consideration of the interindividual variability taking into account potential sensitive subgroups. The IPCS guideline (IPCS, 2005) states that if there are reasons to believe that a specifically sensitive subpopulation can be identified for a given component, the HK_{AF} might rather be computed as the ratio of the upper percentile value of a dose metric in this particularly sensitive subpopulation over the central tendency value in the general healthy, or total, population. The data presented in Table 1 reveal that patients with diabetes, metabolic syndrome, fatty liver or hypertension can be identified as potential sensitive populations, given their higher plasma glutamate levels as compared to healthy subjects. Metabolic syndrome was defined according to the Japanese diagnostic criteria for metabolic syndrome. Subjects with metabolic syndrome had visceral obesity (waist ≥ 85 cm in males and ≥90 cm in females) plus at least 2 of the following three components: (1) HDL-C < 40 mg/dL, TG ≥ 150 mg/dL, or the use of medication for dyslipidemia; (2) FPG ≥ 110 mg/dL or the use of medication for diabetes; and (3) blood pressure ≥ 130/85 mmHg or the use of antihypertensive medication. Diabetes was defined in patients with FPG ≥126 mg/dL, HbA1c ≥ 6.5%, or those who were taking medication for diabetes. Hypertension was defined in patients with blood pressure ≥140/90 mmHg or those who were taking antihypertensive medications. Liver fat content was defined by the ultrasound hepatic/renal ratio. Healthy subjects were defined as previously described (Yamamoto et al., 2016).

The highest plasma glutamate levels were reported for the subgroup with metabolic syndrome (Table 1). Using the GM value of the healthy population and the high percentile values of the subjects with metabolic syndrome to calculate the HK_{AF} values results in the data presented in Tables 5 and 6.

The HK_{AF} values thus obtained are on average 1.33–1.44 fold higher because the glutamate plasma levels in the subgroup with metabolic syndrome are on average 1.33–1.44 fold higher than the levels of the healthy population (Tables 5 and 6). When the plasma or brain-related glutamate levels from the literature data would be increased in the sensitive subpopulation in a way similar to what is observed for the data in the Mitsui Memorial Hospital study this would imply that the median HK_{AF} values derived from the literature derived plasma or brain-related glutamate levels would also be 1.33–1.44 times higher. Thus, the HK_{AF} values at the 95th, 97.5th and 99th percentile of 1.75, 1.85 and 1.94 derived from plasma levels (Table 3) and of 1.26, 1.30 and 1.37 derived from brain-related levels (Table 4) would amount to values of respectively 2.33–2.52, 2.46–2.66 and 2.58–2.79 for HK_{AF} values derived from plasma data and to 1.68–1.81, 1.73–1.87 and 1.82–1.97 for HK_{AF} values derived from brain-related data.

4. Discussion and conclusions

For nutrients or other macronutrients including amino acids that are also used as food additives the traditional method for establishment of an HBGV by identification of a NOAEL or BMDL from a suitable key study, to which a default “safety” or “uncertainty” factor of 100 is applied, may not be applicable. Daily intakes of these substances in the human population at levels required for normal physiological function are in such quantities that it is generally not possible to apply a default 100-fold safety factor for human intakes. Dosing laboratory animals at levels that could approach 100-fold of normal human dietary intake could result in nutritional imbalances that may yield secondary adverse effects (IPCS/UNEP/WHO, 1987).

The group ADI of 30 mg/kg bw/day recently established by EFSA for glutamate and its salts (EFSA, 2017) illustrates the pitfalls of applying this food additive NOAEL/BMDL-uncertainty factor paradigm to nutrients. The group ADI of 30 mg/kg bw/day sets the HBGV for glutamate below its normal dietary intake, and also below the intake of free glutamate by breast fed babies and babies fed protein hydrolysate infant formula (Roberts et al., 2018). This result indicates the need for an alternative approach to the establishment of an HBGV for macronutrients including glutamate. The need for an alternative approach to the safety assessment of macronutrients has been highlighted before (Rodricks, 2003; Borzelleca, 1992a, 1992b, 1996; Dybing et al., 2002). Recently, also EFSA published a draft statement on the derivation of HBGVs for regulated products that are also nutrients (EFSA, 2020). The EFSA draft statement indicates that when defining a HBGV for nutrients one should keep in mind the concept of an acceptable range of oral intake (AROI) to ascertain establishment of an HBGV within the boundaries of rising risks related to deficiency or toxicity. An AROI for glutamate has formally not been defined. However one could argue that the intake of free glutamate by breast fed babies or by babies fed protein hydrolysate infant formula would fall within the AROI. Intake of glutamate from mothers milk by breast fed babies, may amount to levels of 27–32 mg/kg bw per day, as can be calculated based on the level of free glutamate in breast milk reported to amount to 1529 μmol/L (equal to 225 mg/L) (Zhang et al., 2013), and assuming an intake of 600 or 1000 mL by a 2 or 6 month old infant of 5 or 7 kg bw (Roberts et al.,

Table 3
Overview of GM and 95th, 97.5th and 99th percentile values of the distributions created by Monte Carlo simulation of literature data and data from the present study on human plasma glutamate levels, and the HK_{AF} values derived from these data.

Reference	Population	No of subjects	Plasma glutamate level (μmol/L)				HK_{AF}[a]		
			GM	95th	97.5th	99th	95th	97.5th	99th
Present study	Subjects with metabolic syndrome	1000	67.53	108.82	115.47	127.22	1.61	1.71	1.88
	Subjects with diabetes	1000	59.70	103.59	110.66	115.84	1.74	1.85	1.94
	Subjects with hypertension	1000	49.06	87.60	95.28	105.83	1.79	1.94	2.16
	Subjects with fatty liver	1000	61.43	102.96	108.77	114.90	1.68	1.77	1.87
	Healthy subjects	1000	39.70	74.43	81.79	88.12	1.87	2.06	2.22
Shimmura et al. (2011)	Healthy male control children	1000	20.34	27.53	29.45	30.47	1.35	1.45	1.50
	Male children with high functioning autism (HFA)	1000	26.92	41.29	43.99	46.22	1.53	1.63	1.72
Makhro et al. (2016)	Healthy untrained volunteers before exercise	1000	35.89	50.45	53.15	55.55	1.41	1.48	1.55
	Healthy untrained volunteers after exercise	1000	48.49	80.72	85.72	91.25	1.66	1.77	1.88
	Endurance athletes before exercise	1000	32.97	54.09	58.73	63.72	1.64	1.78	1.93
	Endurance athletes after exercise	1000	43.68	59.59	60.65	62.44	1.36	1.39	1.43
Droge et al. (1988)	Apparently healthy persons	1000	54.29	80.48	84.43	88.00	1.48	1.56	1.62
Schmidt et al. (2016)	Meat-eaters	1000	261.09	273.12	276.11	279.23	1.05	1.06	1.07
	Fish-eaters	1000	296.24	309.18	312.22	314.62	1.04	1.05	1.06
	Vegetarians	1000	276.47	289.04	290.91	292.69	1.05	1.05	1.06
	Vegans	1000	262.05	275.17	277.57	280.47	1.05	1.06	1.07
Nakamura et al. (2016)	Japanese	1000	24.26	46.63	52.09	54.65	1.92	2.15	2.25
	Korean	1000	20.42	43.39	47.43	53.67	2.12	2.32	2.63
	Chinese	1000	30.00	68.71	76.91	87.72	2.29	2.56	2.92
Kamada et al. (2016)	Healthy subjects	1000	28.25	59.44	65.41	70.56	2.10	2.32	2.50
Janjua et al. (1992)	Controls	1000	19.25	27.77	28.82	31.02	1.44	1.50	1.61
Reis et al. (2006)	Healthy Controls	1000	164.85	224.09	236.13	247.57	1.36	1.43	1.50
Ortega et al. (2003)	Control [non-pregnant woman]	1000	47.37	92.61	98.15	107.30	1.96	2.07	2.27
	Pregnant woman gestational age: $\leq$ 32 weeks	1000	38.47	70.80	74.33	82.40	1.84	1.93	2.14
	Pregnant woman gestational age: $>$ 32 weeks	1000	59.80	109.09	119.04	130.19	1.82	1.99	2.18
Butte et al. (1999)	GDM [fasting] Antepartum	1000	66.50	92.14	97.14	100.62	1.39	1.46	1.51
	GDM [fasting] Postpartum	1000	62.04	110.44	117.24	122.33	1.78	1.89	1.97
	Control [fasting] Antepartum	1000	49.78	123.77	134.98	145.58	2.49	2.71	2.92
	Control [fasting] Postpartum	1000	34.97	56.83	60.99	64.56	1.63	1.74	1.85
Posod et al. (2017)	Term group	1000	89.78	157.33	164.60	172.34	1.75	1.83	1.92
	Preterm group	1000	36.51	71.98	79.11	84.24	1.97	2.17	2.31
Chuang et al. (2002)	Age group: 0–24 months	1000	47.15	117.01	123.73	127.72	2.48	2.62	2.71
	Age group: 2–18 years	1000	33.59	74.24	78.90	87.41	2.21	2.35	2.60
	Age group: 19–68 years	1000	24.90	57.44	62.92	70.29	2.31	2.53	2.82
	Age group: 0–3 months	1000	64.01	125.71	140.93	142.38	1.96	2.20	2.22
	Age group: 4–24 months	1000	51.43	112.96	123.42	134.53	2.20	2.40	2.62
Perry et al. (1975)	Healthy persons	1000	20.20	40.52	43.26	47.66	2.00	2.14	2.36
Median							1.75	1.85	1.94

[a] HK_{AF} is calculated as the glutamate plasma level at a given percentile of the distribution divided by the GM created by Monte Carlo simulation (IPCS 2005).

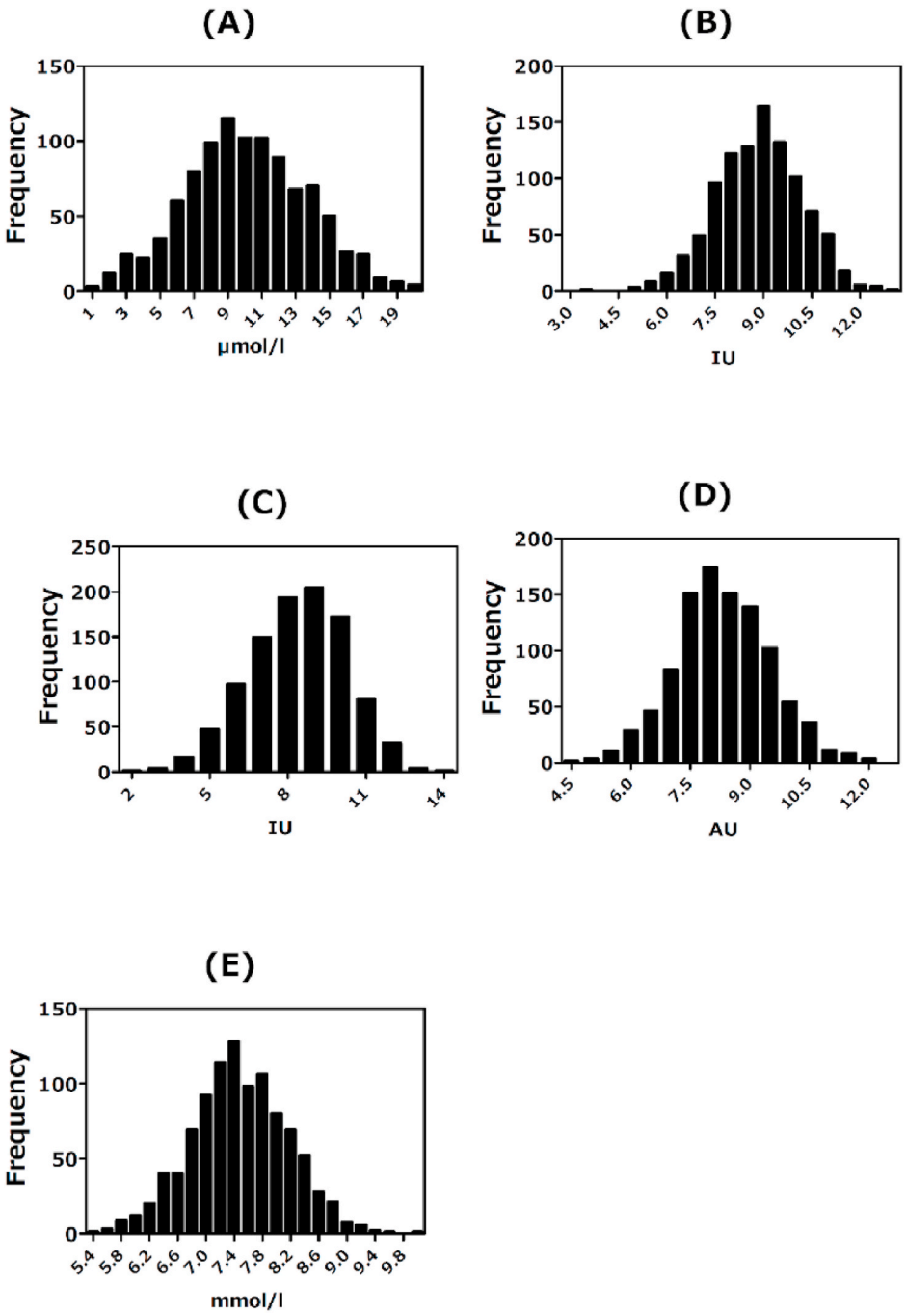

Fig. 4. Monte Carlo simulation based distributions of brain-related glutamate levels in tissues of subgroups (N = 1000 in each group) of subjects including A) cerebrospinal fluid of adult subjects (Perry et al., 1975), B) right striatum of healthy non-obese humans (Caravaggio et al., 2018), C) left striatum of healthy non-obese humans (Caravaggio et al., 2018), D) hippocampus of healthy controls (Bossong et al., 2019), and E) anterior cingulate cortex of healthy controls (Hoerst et al., 2010).

2018). Protein hydrolysate infant formula contains much more glutamate than breast milk (Ventura et al., 2012). A survey of the free amino acid content in protein hydrolysate infant formula revealed the presence of glutamate at concentrations of up to over 8000 µmol/L (equal to about 1200 mg/L). Using the same assumptions this would result in intakes amounting to about 144–171 mg/kg bw per day (Roberts et al., 2018). These estimates do give some directions to what an AROI for glutamate could be. The group ADI of 30 mg/kg bw/day for glutamates appears to be outside this AROI. The concept of an AROI is based on the consideration that nutrients have distinctive physiological roles and kinetic processes that maintain homeostasis over a range of intakes (EFSA, 2020). This concept of homeostasis already supports that the use of the default uncertainty factor of 3.16 for differences in human kinetics can be unnecessary conservative and even inadequate for nutrients. An alternative approach may include the use of chemical-specific adjustment factors replacing the default uncertainty factor of 100, an approach recently used by EFSA to define the ADI for phosphates (EFSA, 2019). The present paper investigated the definition of a HK_{AF}, a CSAF for human interindividual differences in kinetics, using data sets on plasma glutamate levels available for subgroups of subjects in Mitsui Memorial Hospital and on plasma and brain-related glutamate levels as reported in the literature for different groups of subjects.

The experimental data on plasma glutamate levels for different subgroups of subjects in Mitsui Memorial Hospital were from subjects enrolled in the Ningen Dock comprehensive medical check-up system at Mitsui Memorial Hospital. As a general condition of the check-up system, all of the subjects were fasted. For most of the literature data subjects were non-fasted. However, base values of glutamate plasma

Table 4
Overview of GM and 95th, 97.5th and 99th percentile values of the distributions created by Monte Carlo simulation of literature data on brain-related glutamate level, and the HK_{AF} values derived from these data.

Reference	Sample/Unit of Glutamate	Population	No of subjects[a]	Glutamate level				HK_{AF}[b]		
				GM	95th	97.5th	99th	95th	97.5th	99th
Perry et al. (1975)	Cerebrospinal Fluid of adult subjects/μmol/L	Healthy persons	1000 (43)	1.64	3.04	3.23	3.54	1.85	1.97	2.15
Caravaggio et al. (2018)	Right striatum of healthy non-obese humans/IU	Healthy non-obese humans	1000 (17)	8.75	10.95	11.29	11.78	1.25	1.29	1.35
	Left striatum of healthy non-obese humans/IU	Healthy non-obese humans	1000 (17)	8.14	11.20	11.70	12.25	1.38	1.44	1.51
Bossong et al. (2019)	Hippocampus of healthy controls/arbitrary units (AU)	Healthy Controls	1000 (30)	8.22	10.34	10.67	11.27	1.26	1.30	1.37
Hoerst et al. (2010)	Anterior cingulate cortex of healthy controls/mmol/L	Healthy Controls	1000 (30)	7.44	8.66	8.83	9.08	1.16	1.19	1.22
Median								1.26	1.30	1.37

[a] The number in parenthesis is the number of subjects included in the study extracted from the literature.
[b] HK_{AF} is defined as the brain-related glutamate level at a given percentile of the distribution created by Monte Carlo simulation divided by the GM (IPCS 2005).

Table 5
HK_{AF} values derived from the data from Mitsui Memorial Hospital presented in Table 1 taking the subjects with metabolic syndrome as a sensitive subpopulation.

Population	No of subjects	Plasma glutamate level (μmol/L)				HK_{AF}a		
		GM	95th	97.5th	99th	95th	97.5th	99th
Subjects with metabolic syndrome	131	66.55	107.67	120.68	130.15	2.62	2.94	3.17
Healthy subjects	526	41.07	80.02	90.00	99.56			
Relative increase in plasma glutamate plasma level in subjects with metabolic syndrome			1.35	1.34	1.31			

[a]HK_{AF} is calculated as the glutamate plasma level at a given percentile of the distribution for the subjects with metabolic syndrome divided by the GM for healthy subjects (IPCS, 2005).

Table 6
HK_{AF} values derived from the data from Mitsui Memorial Hospital presented in Table 2 taking the subjects with metabolic syndrome as a sensitive subpopulation.

Population	No of subjects	Plasma glutamate level (μmol/L)				HK_{AF}[a]		
		GM	95th	97.5th	99th	95th	97.5th	99th
Subjects with metabolic syndrome	1000	67.53	108.82	115.47	127.22	2.74	2.91	3.20
Healthy subjects	1000	39.70	74.43	81.79	88.12			
Relative increase in glutamate plasma level in subjects with metabolic syndrome			1.46	1.41	1.44			

[a] HK_{AF} is calculated as the glutamate plasma level at a given percentile of the distribution for the subjects with metabolic syndrome divided by the GM for healthy subjects (IPCS, 2005).

levels are known not to be affected following even a high intake of dietary glutamate (Tsai and Huang, 1999), supporting definition of HK_{AF} values irrespective of the fact of whether subjects were fasted or not. Given this consideration, in the present study the HK_{AF} values were calculated based on plasma glutamate levels as reported, without further correction for potential differences in glutamate intake. The assumption that glutamate intake does not affect the compound specific HK_{AF} is supported by the fact that HK_{AF} values derived from the plasma glutamate levels for the different groups of subjects at Mitsui Memorial Hospital who were all fasted, are comparable to the HK_{AF} values derived from study populations from literature who were not fasted. Another aspect to consider is that the experimental data presented in the current study were derived from subpopulations of Japanese subjects, and in some cases relate to subjects under medical supervision. However, glutamate plasma levels derived from these subjects and from a wide range of different subgroups used in the different literature studies, including subjects on different diets, children, pregnant women and subjects from different ethnic backgrounds, all resulted in comparable HK_{AF} values. This may be explained by the fact that glutamate is a normal endogenous metabolite under strict homeostatic control, and further supports the limited interindividual variability in human glutamate kinetics. Although the IPCS guidelines indicate that the use of a CSAF does not require data from multiple studies in humans if suitable data of sufficient quality are available from a single study (IPCS, 2005), the present study presents a HK_{AF} value for glutamate based on a wide range of studies and study subjects.

The HK_{AF} values in the present study were obtained by using the data as presented in the different publications on an individual basis, but also by using a Monte Carlo simulation to define the distributions for somewhat larger populations. Use of the Monte Carlo simulation also allowed analysis of literature data that only reported mean or geometric mean values for human plasma or brain-related levels of glutamate and their SD. All HK_{AF} values obtained were lower than the default value of 3.16. The median HK_{AF} values obtained based on plasma glutamate levels by the two approaches do not vary substantially and amount to 1.64 and 1.75 at the 95th percentile, 1.75 and 1.85 at the 97.5th percentile and 1.82 and 1.94 at the 99th percentile, when using reported distributions or using the Monte Carlo simulations, respectively. For data sets where both analyses were feasible the distributions obtained using the Monte Carlo modelling showed somewhat wider distributions resulting in slightly higher HK_{AF} values. This indicates the Monte Carlo approach to be more conservative. The median HK_{AF} values derived using Monte Carlo simulations from the available data on human brain-related glutamate levels were lower than those obtained from human plasma data amounting to 1.26 at the 95th percentile, 1.30 at the 97.5th percentile and 1.37 at the 99th percentile. Overall these HK_{AF} values point at limited interindividual differences in glutamate kinetics within the human population. Such limited interindividual differences in glutamate plasma levels may be related to the homeostatic control, which is known to maintain extracellular glutamate concentrations at low levels in both brain and peripheral tissues by sodium-dependent glutamate transporters (Hawkins, 2009; Kanai et al., 2013).

It is also of interest to note that the HK_{AF} values obtained based on glutamate levels in plasma seem to adequately reflect variability in the brain as target tissue for glutamate induced effects. The somewhat lower HK_{AF} values derived based on the brain-related glutamate levels indicate

the variability in the target tissue to be even smaller than that in plasma of the study subjects.

Given that the data from the study performed at Mitsui Memorial Hospital relate to different subgroups within the population, analysed within the same study, they enable consideration of the interindividual variability taking into account also potentially sensitive subgroups. The highest plasma glutamate levels were reported for the subgroup with metabolic syndrome. Glutamate levels in this subgroup are apparently no longer under the normal homeostatic control, although within the subgroup interindividual variability is comparable to that within healthy subjects. The physiological reason why plasma glutamate levels in the metabolic syndrome group could be higher than those in the healthy group may be related to the fact that insulin resistance induces glutamate dehydrogenase (GDH) activity, resulting in higher plasma glutamate levels (Stanley, 2009; Maltais-Payette et al., 2019). The HK_{AF} values obtained taking this sensitive subgroup into account were 1.33–1.44 fold higher. Assuming that the relative increase in literature reported plasma levels and brain-related glutamate levels in a sensitive subgroup would be similar, the HK_{AF} values derived from these data would then also be 1.33–1.44 fold higher. Thus they would amount at the 95th, 97.5th and 99th percentile to values of respectively 2.33–2.52, 2.46–2.66 and 2.58–2.79 for HK_{AF} values derived from plasma data and to 1.68–1.81, 1.73–1.87 and 1.82–1.97 for HK_{AF} values derived from brain-related data. Considering the choice of an adequate HK_{AF} for glutamate it is of interest to note that the IPCS indicated that selection of the 95th, 97.5th or 99th percentile as basis for the HK_{AF}, is a matter of expert judgement and a choice to be made by the risk assessor or risk manager on a case-by-case basis (IPCS, 2005). Given that often the 95th percentile is selected in risk assessment to protect sensitive individuals, for example when defining a BMDL, a threshold of toxicological concern (TTC) or to estimate exposure of high level consumers, use of the 95th percentile seems also appropriate for defining the HK_{AF}. In Figure 9 of the guideline the IPCS document explains the estimation of a HK_{AF} for a bimodal population also selecting the 95th percentile. of the sensitive subgroup (IPCS, 2005). Based on these considerations and taking into account the subjects with metabolic syndrome in the Mitsui Memorial Hospital study as a sensitive subpopulation, the HK_{AF} values amount to 2.62 or 2.74. Taking into account an also 1.33–1.44 fold increase for the HK_{AF} values derived from the literature data on plasma and brain-related glutamate levels, the HK_{AF} obtained at the 95th percentile amount to 2.33–2.52, for the plasma data and to 1.68–1.81 for HK_{AF} values derived from brain-related data.

Taking this all together it is concluded that a HK_{AF} of 2 or 2.5 would provide a reasonable value. Combining these values with the previously identified chemical specific adjustment factor for the interspecies differences in glutamate kinetics of 1 (Roberts et al., 2018) and the default factors for interspecies and interindividual differences in dynamics of 2.5 and 3.16 (IPCS, 2005) results in an overall CSAF for glutamates of 16–20 to replace the default value of 100. Use of a CSAF of 16–20 together with the point of departure from the 3-generation reproductive toxicity study (Anantharaman, 1979) providing a NOAEL for neurodevelopmental toxicity of at least 6000 mg/kg bw/day, being the highest dose tested, results in an ADI of 300–375 mg/kg bw/day. Based on this HBGV the high-level combined dietary intake of glutamate from natural occurrence in foods and from use of food additives, which were both estimated to be around 80 mg/kg bw/day (Tennant, 2018) would no longer exceed the ADI. This also holds for the intake estimates from natural and added sources reporting average intakes that ranged from 11 mg/kg bw/day (Danish elderly) to 74 mg/kg bw/day (toddlers Belgium), with high level intakes amounting to over 110 mg/kg bw/day for toddlers in Bulgaria and other children in Belgium (Tennant, 2018). Intakes for glutamate via protein hydrolysate infant formula, of 144–171 mg/kg bw per day (Roberts et al., 2018), or the intake estimated for a typical breastfed infant of 27–32 mg/kg bw/day (Roberts et al., 2018) or 40 mg/kg bw at the mean and 70.2 mg/kg bw at the upper range (Koletzko, 2018) would also no longer exceed the ADI.

Altogether it is concluded that using a CSAF to define an HBGV for glutamate would result in a value that is no longer below the AROI.

CRediT authorship contribution statement

Ivonne MCM. Rietjens: Conceptualization, Investigation, Methodology, Formal analysis, Investigation, Project administration, Writing - original draft, preparation, Writing - review & editing, Supervision. **Takayuki Tanaka:** Conceptualization, Investigation, Methodology, Software, Validation, Visualization, Formal analysis, Writing - original draft, preparation, Writing - review & editing, Supervision. **Yoko Masuzawa:** Conceptualization, Funding acquisition, Resources, Project administration, Investigation, Supervision. **Hidehiro Nakamura:** Designing clinical research, conducting clinical research, Writing - review & editing. **Yuko Ishizaka:** Designing clinical research, conducting clinical research, Writing - review & editing. **Keng Ngee Teoh:** Conceptualization, Funding acquisition, Resources, Project administration, Investigation, Methodology, Writing - review & editing, Supervision, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

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