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Food supplements - High intake of isolated branched-chain amino acids can lead to health impairments

BfR Opinion No. 052/2019 of 20 December 2019¹

Leucine, isoleucine and valine form the group of branched-chain amino acids (BCAAs²). As protein building blocks, they are natural components of foods containing protein such as meat, fish or legumes. In the context of a normal diet, BCAAs are always ingested in combination with other amino acids. BCAAs can be extracted from protein sources and can be consumed in isolated form, as single BCAA or as combination of all three BCAAs or only of two of them, for example in the form of certain food supplements. However, there are indications of possible health risks associated with higher intake levels of BCAAs in isolated form. These particularly relate to undesirable changes in laboratory parameters, for example increased blood ammonia levels. The German Federal Institute for Risk Assessment (BfR) has taken this as a reason to derive guidance values for tolerable supplemental daily intakes of leucine, isoleucine and valine in isolated form, when ingested as a single BCAA or as combination of the BCAAs).



The available scientific human data are currently insufficient to derive these guidance values. Therefore, data obtained from toxicological animal studies with these amino acids were used. On the basis of the highest daily intake levels, where no toxic effects were observed (No Observed Adverse Effect Level or short NOAEL) identified in these studies, and taking into account uncertainty factors for the extrapolation of these toxicological parameters determined in animal studies to humans, the BfR has derived the following guidance values for adults. They apply to the intake of isolated branched-chain amino acids (as single BCAA or combination of the BCAAs), which can be consumed daily in addition to the normal diet:

Leucine:	4.0 g per day
Isoleucine:	2.2 g per day
Valine:	2.0 g per day
BCAAs (total):	8.2 g per day (corresponding to the sum of the guidance values for the individual BCAAs)

The available findings also suggest that branched-chain amino acids should not be taken individually, but in combination of all three BCAAs. Due to insufficient data, it is currently not possible to derive guidance values for the tolerable supplemental daily intake of BCAAs in isolated form for children, adolescents, pregnant and breast-feeding women. The BfR therefore recommends that these population groups avoid relevant intakes of isolated branched-chain amino acids, for example from food supplements or sports foods. In addition, the derived guidance values do not apply to individuals with reduced kidney function and those who follow a diet with a low protein intake. These individuals should consult their physician before consuming relevant isolated amounts of these amino acids.

¹ Please note: A preliminary version of this opinion was sent to the Federal Ministry of Food and Agriculture (BMEL) in December 2017 and reflects the current state of scientific knowledge at that time.

² BCAA = Branched-chain amino acid
BCAAs = Branched chain amino acids

 BfR risk profile: Addition of branched-chain amino acids (BCAAs) in isolated form to food (Opinion No. 052/2019)	
A Affected persons	General population 
B Probability of health impairment with high supplemental intakes of isolated branched-chain amino acids, as single BCAA or combination of the BCAAs	Practically impossible Unlikely Possible Probable Certain
C Severity of health impairment with high supplemental intakes of isolated branched-chain amino acids, as single BCAA individually or combination of the BCAAs	No data available
D Validity of available data	High: The most important data is available and there are no contradictions Medium: Some important data is missing or contradictory Low: Many important data are missing or contradictory
E Controllability by the consumer	Control not necessary Controllable through precautionary measures Controllable through avoidance Not controllable

Fields marked in dark blue indicate the properties of the risk assessed in this opinion (for more details, see the text of Opinion No. 052/2019 of the BfR dated 20 December 2019).

Explanations

The risk profile is intended to visualise the risk outlined in the BfR opinion. The profile is not intended to be used to compare risks. The risk profile should only be read in conjunction with the corresponding opinion.

Row C - Severity of health impairment:

[3] - Based on the available data, the severity of the potential risk cannot currently be quantified.

Line E - Controllability by consumers

[2] - The BfR made recommendations for action in its opinion: For isolated leucine, isoleucine, valine and BCAAs intakes, guidance values for tolerable supplemental daily intake for adults were derived. The BfR recommends that children, adolescents, pregnant women and nursing mothers avoid relevant intakes of isolated branched-chain amino acids, for example from food supplements or sports foods. In addition, the derived guidance values do not apply to individuals with reduced kidney function and those who follow a diet with a low protein intake. These individuals should consult their physician before consuming relevant isolated amounts of branched-chain amino acids.

1 Subject of the assessment

The German Federal Institute for Risk Assessment (BfR) has conducted a risk assessment of the isolated intake or the isolated addition, respectively, of the three branched-chain amino acids L-leucine, L-isoleucine and L-valine, individually or in combination, i.e. ingested as single BCAA or in combination of two or all three BCAAs, but without simultaneous addition of other essential and non-essential amino acids. The assessment concerns the addition of the branched-chain amino acids to foods for general consumption and only the addition of the L-forms of the amino acids (in the following, therefore, the amino acids mentioned refer to their L-form).

The three branched-chain amino acids are often used in combination. In the following, the combined use of leucine, isoleucine and valine is referred to as BCAAs administration or addition (BCAA = **branched-chain amino acid**), whereby the quantitative ratios of the three amino acids often vary in available human studies, however, the respective quantitative proportions of leucine are usually higher than those of isoleucine or valine.

2 Results

In the risk assessment of isolated leucine, isoleucine and valine intakes, as single BCAA or as combination of the BCAAs (= BCAAs administration), considerable scientific uncertainties exist due to insufficient data. However, from the available scientific studies there are indications of possible health risks from higher intakes of branched-chain amino acids, as single BCAA or combination of the BCAAs, which currently primarily concern undesirable changes in laboratory parameters (e.g. ammonia blood levels). Due to the insufficient scientific data, questions also arise as to whether and to what extent there are undetected health risks associated with high isolated leucine, isoleucine, valine and BCAAs intakes.

The data currently available are not sufficient for any of the amino acids to be assessed (either leucine, isoleucine, valine or the combined administration of the branched-chain amino acids) to derive the lowest daily intake, exceedance of which would be expected to have harmful health effects.

In view of available indications on possible health risks of higher isolated leucine, isoleucine and valine or BCAAs intakes and the existing scientific uncertainties in the risk assessment of branched-chain amino acids, it seems appropriate to derive health based guidance values. For isolated leucine, isoleucine, valine and BCAAs intakes, the BfR has therefore derived guidance values for tolerable supplemental daily intakes, i.e. daily intakes that can be ingested in addition to the intake via the usual diet and which, based on current knowledge, are likely to pose no or only a very low risk of undesirable effects.

Due to insufficient scientific data, the available human studies are not suitable for the derivation of guidance values for tolerable supplemental daily intakes for isolated leucine, isoleucine, valine or BCAAs doses. Animal studies to investigate the sub-chronic or chronic toxicity of these amino acids were therefore used to derive the guidance values. On the basis of No Observed Adverse Effect Levels (NOAEL) identified there, and taking into account uncertainty factors and “default values”³ which are based on recommendations from the European Food Safety Authority (EFSA) (2012), the following guidance values for tolerable supplemental isolated daily intakes for adults were derived:

- Leucine: 4.0 g per day⁴,
- Isoleucine: 2.2 g per day⁵,

³ a) Uncertainty factor of 2 for the extrapolation from sub-chronic to chronic exposure (an uncertainty factor for only sub-chronic exposure appears justified, given the findings for isoleucine, where adverse effects were observed during chronic exposure, that were not apparent during sub-chronic exposure),

b) An uncertainty factor of 10 to take into account interspecies variability,

c) An uncertainty factor of 3.16, rounded down to 3, to take account of human inter-individual variability (given that these amino acids are essential nutrients, a reduced uncertainty factor seems justified),

d) Body weight (bw) of adults: 70 kilograms (kg)

⁴ Guidance value for the tolerable supplemental daily intake of isolated leucine =

NOAEL (sub-chronic) = 3330 mg per kg bw

Calculation: (3330 mg per kg bw per day: (2 x 10 x 3)) x 70 kg = 3885 mg per day (at 70 kg bw)

⁵ Guidance value for the tolerable supplemental daily intake of isolated isoleucine =

- Valine: 2.0 g per day⁶,
- BCAAs: 8.2 g per day (corresponding to the sum of the guidance values derived for the three individual branched-chain amino acids).

The present findings also suggest that branched-chain amino acids should not be taken individually, but in combination of all three branched-chain amino acids⁷.

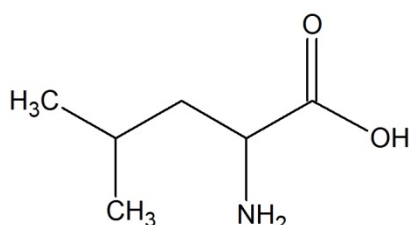
The data currently available are insufficient for a risk assessment of isolated leucine, isoleucine, valine or BCAAs intakes in children, adolescents, pregnant women and breastfeeding mothers. It is therefore recommended to exclude these population groups from intakes of relevant amounts of isolated branched-chain amino acids, consumed either as single BCAA or combination of the BCAAs.

Based on general theoretical considerations, it is recommended that individuals with impaired kidney function and persons who follow a low protein diet should consult their physician before consuming foods with relevant additions of isolated branched-chain amino acids (leucine, isoleucine, valine, BCAAs).

3 Rationale

3.1 Agent

The amino acids leucine, isoleucine and valine form the group of branched-chain amino acids. They are essential for humans and must be supplied through food. As protein building blocks, they are ubiquitous in protein-containing foods.

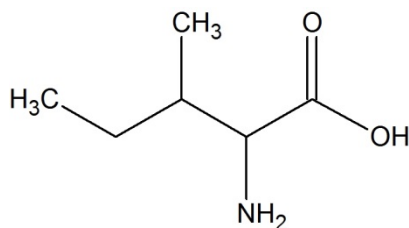


L-leucine, ((S)-2-amino-4-methylpentanoic acid), CAS no.: 61-90-5, empirical formula: C₆H₁₃NO₂, relative molecular weight: 131.17.

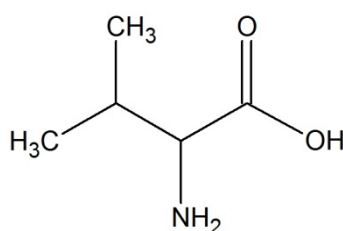
NOAEL (chronic) = 925 mg per kg bw
 Calculation: (925 mg per kg bw per day: (10 x 3)) x 70 kg = 2158 mg per day (at 70 kg bw)

⁶ Guidance value for the tolerable supplemental daily intake of isolated Valine =
 NOAEL (sub-chronic) = 1600 mg per kg bw
 Calculation: (1600 mg per kg bw per day: (2 x 10 x 3)) x 70 kg = 1866 mg per day (at 70 kg bw)

⁷ The ratios of the three branched-chain amino acids can be based on the ratios of the three amino acids, which result from the guidance values derived for leucine, isoleucine and valine, or from the recommended intakes for leucine, isoleucine and valine derived by WHO (2007) or the American FNB (2002/2005).



L-isoleucine, (2S,3S)-2-amino-3-methylpentanoic acid, (2S, 3S)-2-amino-3-methylvaleric acid, CAS no.: 73-32-5, empirical formula: C₆H₁₃NO₂, relative molecular weight: 131.17.



L-valine, (S)-2-amino-3-methylbutyric acid, (S)- α -aminoisovaleric acid, CAS no.: 61-90-5, empirical formula: C₅H₁₁NO₂, relative molecular weight: 117.15.

3.2 Dietary reference values for leucine, isoleucine and valine as essential amino acids

Dietary reference values for the intake of leucine, isoleucine and valine to meet daily needs have been derived from the American Food and Nutrition Board (FNB) and the World Health Organization (WHO). For adults, the daily intake recommendations of the American Board are 42 milligrams (mg) of leucine per kilogram (kg) of body weight (bw), 19 mg of isoleucine per kg bw and 24 mg valine per kg bw⁸ (= 2940 mg leucine, 1330 mg isoleucine and 1680 mg Valine per day at 70 kg bw) (FNB, 2002/2005). Similar recommendations were made by the WHO (39 mg leucine, 20 mg isoleucine and 26 mg valine per kg bw per day (= 2740 mg leucine, 1400 mg isoleucine and 1820 mg valine per day at 70 kg bw) (WHO, 2007).

3.3 Absorption, distribution, metabolism, excretion

The degradation pathways of the three branched-chain amino acids leucine, isoleucine and valine share metabolic similarities. The first two initial steps in their breakdown are carried out by the same enzymes. In humans, these are primarily localized in the extrahepatic tissue (especially skeletal muscles). In both points, the branched-chain amino acids differ from other essential amino acids.

The first step in degradation is a reversible transamination followed by irreversible oxidative decarboxylation in the second step. For all three branched-chain amino acids, these steps are catalysed by the same aminotransferase isoenzymes (branched-chain amino acid aminotransferase) or the same dehydrogenase (branched-chain α -keto acid dehydrogenase), respectively. As a result, the nutritional intake of one branched-chain amino acid can influ-

⁸ Incorrect value for valine in FNB, 2002/2005, page 680.

ence the metabolism of the other two amino acids. Depending on the amino acid, acetyl-coenzyme A, propionyl-coenzyme A, acetoacetate and succinyl-coenzyme A are formed in the further course of degradation. Leucine acts as a ketogenic, valine as a glucogenic and isoleucine as a gluco- and ketogenic amino acid. In the initial transamination reaction, the transfer of the nitrogen group to α -ketoglutarate leads to the formation of glutamic acid. In addition to protein synthesis and as energy substrates, BCAAs serve as nitrogen donors in the brain and peripheral tissues. BCAAs, especially leucine, are important nitrogen sources for the synthesis of the neurotransmitters glutamate and γ -aminobutyric acid in the brain. Leucine may also serve as a signalling agent for lowering protein breakdown (FNB, 2002/2005; Brosnan and Brosnan, 2005; Hutson et al., 2005; Matthews, 2005; Cole, 2015). Leucine is a potent stimulator of the key enzyme mTOR⁹ and the signalling cascade mediated by this protein kinase (Li et al., 2011).

Of the three branched-chain amino acids, leucine appears to be of the greatest importance for human metabolism, but the fact that there is much more scientific knowledge about leucine than about isoleucine and valine may also contribute to this perception.

3.4 Exposure

As protein building blocks, BCAAs are ubiquitous components of protein-containing foods. However, their intake as part of the normal diet takes place in connection with the simultaneous intake of all proteinogenic amino acids. This form of intake must be distinguished from the isolated intake of BCAAs which is subject of this risk assessment.

In the USA, mean daily intakes of 6.1 grams (g) of leucine, 3.6 g of isoleucine and 4 g of valine, corresponding to 13.7 g of BCAAs, were observed across all of the age groups examined. The 95th intake percentiles were highest in 19-30 year old men, at 11.9 g leucine, 6.9 g isoleucine and 7.7 g valine per day or 26.5 g BCAAs per day, respectively (FNB, 2002/2005). The median adult leucine intake in the UK was estimated based on consumption surveys and model calculations at 108 mg per kg bw and the 90th percentile thereof was estimated at 138 mg per kg bw (approx. 7.6 g per day or 9.6 g per day at 70 kg bw, respectively); a daily intake of 200 mg leucine per kg bw (14 g per day at 70 kg bw) was calculated for physically active young men and a daily leucine intake of 262 mg per kg bw corresponding to 23.6 g per day at 90 kg bw was calculated for bodybuilders weighing 90 kg with a high protein intake (Millward, 2012).

3.5 Assessment by scientific bodies or authorities

As was the case for other amino acids, the American Food and Nutrition Board (FNB) of the Institute of Medicine, was unable to derive a tolerable upper intake level (UL) for the intake of isolated branched-chain amino acids in combination or for the intake of single isolated leucine, isoleucine or valine, due to lack of data (FNB 2002/2005).

The *Health Council of the Netherlands*, in its 1999 risk assessment of the use of isolated amino acids in food supplements and fortified foods, agreed with an earlier statement by the *Federation of American Societies for Experimental Biology* (FASEB) (Anderson and Raiten, 1992) that there were not sufficient data available to evaluate the safety of amino acids as

⁹ mTOR = mammalian Target of Rapamycine.

food supplements and no safe upper levels of intake (for the additional supply via supplements) could be derived. However, the panel decided to specify maximum acceptable levels for the addition of individual amino acids to foods including food supplements. These safe upper levels are not based on toxicological derivations but, due to a lack of data, on a general approach. This is based on the intake of the individual amino acids, which result from the (Dutch) recommendations for protein intake for women (0.8 g per kg bw and a body weight of 65 kg) in combination with a reference protein¹⁰. The maximum daily intake quantities derived in this way are 5 g for isolated leucine, 3.1 g for isolated valine and 2.9 g for isolated isoleucine, corresponding to 11 g of isolated BCAAs. The Council recommended not to use branched-chain amino acids individually, but in a combination of all three BCAAs and in the proportions given. In its assessment the panel drew attention to the fact that pregnant women, breastfeeding mothers, children under the age of 13, patients suffering from metabolic disorders, persons taking certain medications and persons who are on a low-protein diet should be discouraged from using additional intakes of amino acids (Health Council of the Netherlands, 1999).

In its 2013 assessment, the *Scientific Committee of the Spanish Agency for Food Safety and Nutrition* concluded among other things: "Moreover, there is no maximum tolerable intake level, as no toxicity studies on humans are available to provide these bases". However, the panel considered a maximum daily quantity of 5.45 g BCAA per day, proposed for dietary supplements, to be acceptable for the sum of the three branched-chain amino acids. (This amount should correspond to the sum of the individual amounts accepted for these three amino acids (3 g leucine per day, 1.5 g isoleucine per day and 1.95 g valine per day), in which case the sum of these three individual amounts adds up to 6.45 g per day). With this maximum daily quantity, the panel believes that warnings that these amino acids should not be taken by pregnant women and children or for prolonged periods of time without medical supervision to be necessary (AESAN, 2013). Among others, the assessment in question makes reference to the results of the study by Zhang et al. (2011) (see 3.7.3.4).

In a recent opinion, the *Norwegian Scientific Committee of Food Safety (VKM)* addressed the risk assessment of BCAA intakes via food supplements, considering intakes of 2.5-5.25 g leucine, 1.5-2.5 g Isoleucine and 1.5-2.5 g of valine per day. The panel concluded that due to the lack of studies addressing the occurrence of undesirable effects with these amounts, no conclusions can be drawn about the health risks of such daily intakes for adults, adolescents or children (VKM, 2016).

In Canada, the addition of branched-chain amino acids to so-called "workout supplements" is limited to 1824 mg leucine, 1065 mg isoleucine and 1194 mg valine (= 4083 mg BCAA) per day (Health Canada, 2019).

In the case of the use of isolated leucine in food, information is available indicating that the *American Food and Drug Administration (FDA)* has raised no objections to two "GRAS"¹¹ No-

¹⁰ Protein intake recommendations for women: 0.8 g protein per kg bw, assumed bodyweight for women: 65 kg, Leucine, isoleucine or valine content of the reference protein (1/3 soy protein and 2/3 casein): 9.4; 5.5 or 6.0 g per 100 g protein,

$$\text{Calculation (leucine): } \frac{0.8 \text{ g} \times 65 \text{ kg} \times 9.4 \text{ g}}{\text{kg} \times 100 \text{ g}} = 4.88 \text{ g}$$

¹¹ GRAS = **G**enerally **R**ecognized **A**s **S**afe.

tices” that concerned the addition of L-leucine obtained from *Escherichia coli* K-12 or of microencapsulated leucine to various foods¹² in the amount of 0.5-3 g of L-leucine per consumption unit. In both cases, the daily intakes resulting from the consumption of the specified enriched foods were estimated at 1.9 g L-leucine on average and 4.1 g for the 90th percentile. For the above application, L-Leucine was classified as “Generally Recognized as Safe” by the notifying companies (FDA, 2010a; b; 2014a; b).

3.6 Findings from the pharmaceutical sector

Within the pharmaceutical sector, the combination of the three branched-chain amino acids is used to treat and prevent brain dysfunction in patients with chronic liver disease (latent/manifest hepatic encephalopathy). The usual daily mean dosage for an approved drug is 0.3 g BCAA per kg bw; i.e. for a person with 70 kg bodyweight, a daily dosage of 10.86 g leucine, 4.35 g isoleucine and 5.82 g valine or 21 g BCAA is stated. According to the product information for this medication, no side effects are expected when used as directed. During pregnancy and lactation, the product should only be used under strict indications, as insufficient data are available. Due to the lack of experience with the medication, it should not be given to children under 2 years of age. The medication must not be used if renal function is impaired (contraindication) (PharmNet.Bund, 2008).

3.7 Hazard characterisation

So far, there is no evidence that the supply of amino acids, including valine, leucine and isoleucine, resulting from the protein content of foods and occurring in combination with all other proteinogenic amino acids represents a health risk. However, this does not apply to the intake of isolated amino acids, e.g. via their addition as isolated amino acids to dietary supplements or enriched foods. This can potentially result in amino acid imbalances.

3.7.1 Animal studies

Overall, when extrapolating results obtained in rats to humans, it should be taken into account that rats have significantly higher enzyme activities than humans in the two key enzymes of the BCAA-degrading metabolic pathway (branched-chain amino acid aminotransferase, branched-chain α -keto acid dehydrogenase) and that differences in the tissue distribution of both enzymes also exist (Suryawan et al., 1998; Hutson et al., 2005). This limits the applicability of data obtained from rats to humans (FNB, 2002/2005).

3.7.1.1 Studies on sub-chronic and chronic toxicity

Leucine

Tsubuku et al. (2004) examined the sub-chronic toxicity of the branched-chain amino acids and administered rats feed with additions of 0, 1.25, 2.5 and 5 % leucine or isoleucine or valine over 13 weeks. For leucine, the authors derived a No Observed Adverse Effect Level (NOAEL) of 5 % leucine in the feed for both sexes, corresponding to a leucine intake of 3.33 g per kg bw per day for male and 3.84 g per kg bw per day for female

¹² Milk and non-milk-based meal replacement, sports and isotonic drinks, vitamin-enriched water, bars as meal replacement.

animals on the basis of the examinations performed (general condition of the animals, ophthalmological examinations, urine analysis, haematological and clinical-chemical examinations, determination of the weights of selected organs and tissues as well as pathological and histopathological examinations).

Isoleucine

In studies examining the sub-chronic toxicity of isoleucine (0, 1.25, 2.5, 5 or 8 % added to the feed) in rats (duration: 13 weeks), various effects were observed with 5 and 8 % isoleucine (e.g. with 8 % isoleucine slight but statistically significant increased relative kidney weights in both sexes and statistically significantly increase in urine volume in male animals; increased urine pH for male animals from 2.5 % isoleucine; slight, but statistically significant changes in some blood biochemical parameters with 5 and 8 % isoleucine). The authors derived a No Observed Effect Level (NOEL) of 2.5 % isoleucine (Kawabe et al., 1996).

In the above mentioned study in rats with the addition of 0, 1.25, 2.5 and 5 % isoleucine in the feed, Tsubuku et al. (2004) derived a No Observed Adverse Effect Level (NOAEL) of 2.5 % isoleucine in feed for both sexes, corresponding to a daily dose of 1.57 g per kg bw in males and 1.65 g per kg bw in females, on the basis of reduced renal electrolyte excretion, increased positive glucose and urobilinogen results in the urine in male and female animals within the 5 % group (most of the deviations were still within the range of the controls) and statistically significant, slightly increased aspartate and alanine aminotransferase plasma levels in female animals within the 5 % group (in the Kawabe study with 5 % and 8 % leucine, these enzyme levels were not increased, but instead reduced). Testicular findings were recorded in one animal from both the 2.5 % (atrophy of the seminiferous tubules) and the 5 % group (cellular interstitial infiltration).

As part of the evaluation of isoleucine as animal feed, EFSA derived a NOAEL of 1 % from a 90-day study on rats (not available to the BfR) which received feed with 0, 0.2, 1 or 5 % isoleucine, corresponding to an intake of 600 mg per kg bw per day¹³. This was based on slightly increased erythrocyte counts and haemoglobin levels in male animals of the 5 % group as well as reduced platelet counts and significantly prolonged oestrus cycles in female animals of the 5 % group, whereby the panel admitted that the toxicological relevance of these findings is questionable (EFSA, 2010).

Chronic toxicity studies of isoleucine with 0, 2.5 or 5 % isoleucine in feed over 104 weeks showed survival rates of 86 %, 82 % and 74 % in males and 84 %, 86 % and 76 % in females. The difference was not statistically significant. Increased relative kidney weights were recorded in male animals of the 5 % group (although, according to the authors, no histopathological changes were observed that suggested kidney toxicity of the substance). In male animals of the 2.5 % and 5 % group, relative testicular weights decreased in a dose-dependent manner and increased testicular atrophy was observed in animals within the 5 % group. The alanine aminotransferase levels in male animals were slightly, but statistically significantly, increased. EFSA derived a NOAEL of 924.7 mg isoleucine per kg bw per day from this study, corresponding to the isoleucine intake in males with 2.5 % isoleucine in feed (EFSA, 2010; Kawabe et al., 2006).

¹³ No gender-differentiated values

Valine

For valine, the above-mentioned study by Tsubuku et al. (2004) derived a NOAEL of 2.5 % for female animals (reduced weight gain in the 5 % group, although the consumption of feed was also lower), corresponding to 1.85 g per kg bw per day and of 5 % for male animals, corresponding to 3.23 g per kg bw per day using doses of 0, 1.25, 2.5 or 5 % valine in the feed.

A study referenced by EFSA panels (not available to the BfR) on the sub-chronic toxicity (duration 13 weeks) of valine gave somewhat differing results, at least in male animals. In the study, 0, 0.2, 1, or 5 % valine in the feed were administered to rats. In male animals, a significant reduction in bodyweight was observed at the highest intake level, which correlated with lower feed consumption. At the highest dose, increased plasma sodium levels were recorded in both sexes with significantly increased alanine aminotransferase activity in females. The panel derived a No Observed Adverse Effect Level (NOAEL) of 1 % valine in the feed for both sexes, corresponding to 666 mg per kg bw per day for female animals and 628 mg per kg bw per day for male animals (EFSA, 2008a).

In summary, in sub-chronic toxicity studies with rats (13-week studies), a NOAEL for leucine of 5 % in the feed, corresponding to 3.33 g per kg bw per day for male and 3.84 g per kg bw per day for female animals, was derived.

Adverse effects were observed for isoleucine in studies on sub-chronic toxicity, as well as in one study on chronic toxicity, with administration of ≥ 5 % leucine in the feed. The effects were not uniform (effects on testes, kidney/kidney function, liver enzymes). From the studies by Tsubuku et al. (2004) and Kawabe et al. (1996; 2006), a NOAEL of 2.5 % isoleucine in feed can be identified. Based on the study on sub-chronic toxicity (13 weeks; Tsubuku et al., 2004), this corresponded to an isoleucine intake of 1.57 g per kg bw per day (m) or 1.65 g per kg bw per day (f), respectively. In the study on chronic toxicity (104 weeks; Kawabe et al., 2006), this corresponded to an intake of 925 mg per kg bw per day.

For valine, inconsistent adverse effects (effects on bodyweight/weight gain, liver enzymes, sodium plasma levels) were observed in two studies on sub-chronic toxicity when 5 % was added to the feed. Even if the clinical relevance of the observed effects is questionable to some degree (e.g. reduced weight gain with reduced consumption of feed, for which detailed information is partially unavailable), the effects were taken as a reason in both studies to identify the next lower administered dose as a NOAEL, in some cases on a gender-specific basis. Since only doses of 1 and 5 % were examined in the EFSA-referenced study and in the study by Tsubuku et al. (2004) no relevant adverse effects were observed with 2.5 % leucine in feed, this dose could alternatively be used for the identification of a NOAEL for valine, corresponding to an intake of 1.85 g per kg bw for female and 1.60 g per kg bw for male animals.

3.7.1.2 Animal studies on reproductive toxicity

Leucine

When leucine (0.2 ml; 1% solution) was injected into the amniotic sac of 9-day-old chicken embryos or administered intraperitoneally to pregnant rats (15 mg per kg bw),

teratogenic effects of the amino acid were observed (Bergström et al., 1967; Persaud, 1969). 86% of the hatched chicks showed morphological abnormalities compared to 13% of the control animals (Bergström et al., 1967). In rats given intraperitoneal doses from 1st to 6th day of gestation, 50.6% of the foetuses showed abnormalities. In animals that received leucine from the 6th to 9th day of gestation, the rate of foetal absorption was 70.5% (4.4% in control) and the rate of abnormality in fully developed foetuses was 46.2% (Persaud, 1969). However, the relevance of these findings with respect to oral leucine intake is questionable.

With oral daily doses of 300 or 1000 mg leucine per kg bw from 7th to 17th day of gestation (organogenesis) in rats, skeletal variations were recorded in the control group in 11 foetuses from 6 dams, in 13 foetuses from 7 dams within the 300 mg group and in 15 foetuses from 12 dams within the 1000 mg group. Overall, the authors concluded from the study results that with both doses no negative effects were observed on the parameters litter size, weights of the living foetuses, number of *corpora lutea*, implantation index, quality of placenta, gender ratio of the foetuses and on the occurrence of externally visible abnormalities and that no relevant substance-related visceral or skeletal anomalies were observed (Mawatari et al., 2004).

When administering 2 % leucine in combination with 4 % sucrose in drinking water to pregnant rats from the 13th to the 20th day of gestation (roughly approx. 2400 mg leucine per kg bw per day), the foetuses exhibited increased bodyweights and increased absolute pancreatic weights associated with a reduced relative β cell mass and significantly increased foetal blood glucose and significantly reduced insulin plasma levels compared to the control group, which had only received 4% sucrose in the drinking water. Additional *in-vitro* examinations indicate an inhibition of the differentiation of endocrine pancreatic progenitor cells. According to the authors, the findings indicate that leucine supplementation during pregnancy could possibly increase the risk of the occurrence of type 2 diabetes mellitus in the offspring (Rachdi et al., 2012).

Isoleucine

In a study reported by EFSA (2010) (not available to the BfR), rats of both sexes were given feed with 0, 1, 2, 4 or 5 % isoleucine for 14 days before mating. No substance-related effects on fertility, reproductive performance or litter size were observed. However, in the highest dose group (equivalent to 2720 or 4280 mg per kg bw in male or female animals, respectively), significantly more pups were classified as runts. In contrast to the study authors, EFSA therefore came to the conclusion that the highest dose could not be considered to be fully tolerated during the reproductive cycle.

In another study reported by EFSA (2010) (not available to the BfR), 0, 0.2, 1 or 5 % isoleucine in feed was given to rats from mating until the 21st day of gestation. Aside from a temporarily decreased feed intake in the highest dose group, no substance-related effects on the examined parameters were observed. The doses corresponded to intakes of 0, 90-150, 480-750 and 2320-3640 mg isoleucine per kg bw. No detailed information is available on either study (EFSA, 2010).

Valine

In a reproductive toxicology study reported by EFSA panels (not available to the BfR), 0, 0.2, 1.0 or 5 % valine was given in feed to female rats from conception until the 20th day of gestation; in the highest feed group, lower weight gains were observed in the dams on

two measurement days and the weights of the foetuses were significantly lower than in the control group. Referring to the effects observed in the dams at the highest dose (5% valine), the panel concluded that the addition of 1% valine, corresponding to 610 mg per kg bw per day, did not produce any undesirable effects (EFSA, 2008a).

BCAAs (combined administration of the three branched-chain amino acids)

When rats were given feed containing 10 g of leucine plus 10 g of isoleucine and 10 g of valine per kg over 3 generations beginning 14 days before mating, no statistically significant deviations in BCAAs plasma levels compared to the control group were observed in the plasma of the 5-day-old offspring of the F1-F3 generations. However, the dams exhibited increased BCAA levels and reduced tryptophan and tyrosine levels after 14 days of BCAAs administration (no further data at later times). The concentrations of various putative neurotransmitters in the brain stem differed from those of the control group among young, suckling animals of 5-20 days of age in all generations, most clearly in aspartate on day 5 (data for younger animals not available). Brain growth in the F1 generation lagged behind that of the control animals on the 5th and 10th day (approx. 90% of the control values), but was slightly increased on the 20th day. In the F2 and F3 generation, brain weights from day 5 to day 20 were significantly, albeit only slightly reduced, compared to those of the control animals; most pronounced among the F2 generation on the 10th day (approx. 85% of the control value) (Thoemke and Huether, 1984). However, it remains unclear whether these data relate to the absolute or relative brain weights. This limits the scientific informative value of the publication. The observed differences indicate that high maternal BCAA doses during pregnancy and breastfeeding could cause adaptation processes and changes in the balance of metabolic processes in the course of brain maturation of the offspring. However, the functional relevance of the observed differences and changes is unclear. The dose administered in the study was estimated to be about 2250 mg BCAAs per kg bw per day, corresponding to 750 mg per kg bw per day per branched-chain amino acid (FNB 2002/2005).

3.7.2 Studies on mutagenicity and carcinogenicity

Leucine

L-leucine was evaluated by EFSA along with a number of other amino acids in 2008 (EFSA, 2008b). Two *in vitro* genotoxicity studies were considered for L-leucine. A bacterial mutagenicity test with *E. coli uvrB* and *uvrB umuC* strains showed a negative result (Sargentini and Smith, 1986). However, only one dose (2 mM) was tested without the use of a metabolising system (S9 mix) and positive controls are missing. On the other hand, a test with human lymphocytes (sister chromatid exchange assay) showed weak genotoxic effects without the use of a metabolising system (S9 mix) (Xing and Na, 1996). This result was classified as ambiguous by EFSA because the cytotoxicity was not tested and the observed effect showed no dose dependency. Overall, EFSA concluded that the available data did not give rise to safety concerns regarding the genotoxicity of the group of 19 amino acids (including leucine) assessed by JECFA¹⁴ and related substances ("For the substances in this group of 19 JECFA evaluated amino acids and related substances the available data does not give rise to safety concern with respect to genotoxicity.") (EFSA, 2008b).

¹⁴ JECFA = Joint FAO/WHO Expert Committee on Food Additives.

Isoleucine

In vitro studies on mutagenicity (OECD Guideline 471) in four *Salmonella Typhimurium* strains or *Escherichia coli WP2uvrA* with and without metabolic activation (62-5000 micrograms (μg) per plate) and studies on gene mutation in mouse lymphoma cells (0.076-1.25 mg per millilitre (ml)) showed no evidence of isoleucine mutagenicity. The substance proved to be non-clastogenic in the chromosome aberration test (Chinese hamster ovary cells (CHO cells), dose up to 1.31 mg per ml) (EFSA, 2010).

In animal chronic toxicity and carcinogenicity studies on isoleucine, no significant effects on tumour incidence were observed after administration of 0, 2.5 or 5% isoleucine in feed over 104 weeks (however, other adverse effects were observed with the highest intake; see above). (Kawabe et al., 2006; EFSA, 2010).

Valine

Valine was not mutagenic in the Ames test in studies in four *Salmonella Typhimurium* strains and *Escherichia coli WP2uvrA* with and without metabolic activation (62-5000 μg per plate). In the chromosome aberration test (CHO cells, 25-1172 μg per ml) with and without metabolic activation, slight effects on the mitosis index were observed at the highest dose, but this was insufficient to justify the exclusion of this test dose. At the three highest doses, there was no evidence of an increased aberration rate. Valine was therefore classified as not clastogenic under the test conditions used (EFSA, 2008a).

Tumour-promoting effects (leucine and isoleucine)

In animal studies with rats, long-term high isoleucine and leucine intakes can act as promoters in the development of bladder tumours. When 2 % leucine or 2 % isoleucine was given in feed to rats, which had previously been exposed to a temporary administration of a known trigger of bladder cancer (N-butyl-N-(4-hydroxybutyl)nitrosamine; BHBN) at a low dose, an increased incidence and number of bladder carcinomas was observed after 40 or 60 weeks of intake when compared to sole administration of the initiator¹⁵. The administration of isoleucine or leucine alone without prior exposure to BHBN did not lead to the development of bladder cancer (Kakizoe et al., 1983; Nishio et al., 1986).

Another animal study showed that the tumour-promoting effect of the administration of 2 % leucine or isoleucine in feed after BHBN administration is strongly influenced by the type of basal diet given to rats (Xie et al., 2012).

According to the American *Food and Nutrition Board* (FNB), the tumour promotion data obtained in rats cannot be used reliably to assess human risk (FNB, 2002/2005).

In animal models with implanted tumours, administration of the branched-chain amino acids had different effects. In mice with implanted pancreatic tumours, increased tumour growth was observed with calorie-restricted or high-calorie diets supplemented with 5%

¹⁵ After 40 weeks (Study 1): statistically significant increase of incidence with 2% isoleucine administration, the increase with leucine administration did not reach statistical significance ($p < 0.05$),
After 60 weeks (Study 2): statistically significant increase of incidence at 4% isoleucine, the increase with 2% isoleucine did not reach statistical significance; a statistically significant increase of incidence occurred with 2% and 4% leucine.

leucine (Liu et al., 2014). In contrast, a significant reduction in tumour growth was recorded in mice with the implantation of a cachexia-inducing tumour and administration of 1 g leucine or 1 g valine per kg bw per day (Eley et al., 2007). The effects of high doses of branched-chain amino acids on existing tumours may depend on the type of tumour and the animal model used. The relevance of such findings to humans is unclear.

3.7.3 Human studies

Studies on the health effects of isolated intakes of leucine, isoleucine and valine in humans have been carried out in healthy people (mostly athletes) and people suffering from various diseases (e.g. amyotrophic lateral sclerosis, Duchenne muscular dystrophy, tardive dyskinesia, phenylketonuria or severe liver diseases). The amino acids were mostly used in a combination of the three branched-chain amino acids, but also as single BCAA, in which case leucine was mostly used.

It should be noted that there are only a few studies on health effects of long-term intakes of branched-chain amino acids which were administered, as single BCAA or combination of the BCAAs, in healthy individuals. In existing human intervention studies, safety-related aspects, such as the recording of undesirable effects or targeted examinations regarding the occurrence of undesirable effects (e.g. laboratory tests), and their evaluation in the study design or in the publication of study results were not or only insufficiently taken into account.

3.7.3.1 Leucine

Leucine: Effects on plasma levels of branched-chain and other amino acids

Oral single doses of 2 g leucine were able to temporarily reduce valine and isoleucine plasma levels (measurements after 2 and 4 hours). However, no placebo controls were performed in these studies. This limits the informative value of the study. When 10 g of leucine was added, the effect mentioned was enhanced. With single doses of 2 or 10 g leucine, the levels of other essential amino acids showed a tendency to decrease, with methionine and threonine plasma levels as well as (jointly determined) phenylalanine and tyrosine plasma levels being significantly reduced after two hours with 10 g leucine (Swendseid et al., 1965).

In two further studies with single leucine doses¹⁶ of 150-750 mg per kg bw and 150-1250 mg per kg bw (corresponding to 10.5-52.5 and 10.5-87.5 g leucine at 70 kg bw respectively), significant decreases in isoleucine and valine levels were observed at the end of the study day compared to an intake of 50 mg leucine per kg bw (= 3.5 g leucine at 70 kg bw; this dose roughly corresponds to the recommended intake for leucine; see Chapter 3.2) (Elango et al., 2012; Rasmussen et al., 2016).

¹⁶ In each case, the leucine doses were administered on one day only, with the respective daily doses being administered in the form of several individual doses distributed over the day.

In patients who suffered from a form of multi-system atrophy (glutamate dehydrogenase-deficient olivopontocerebellar atrophy), oral doses of 100-150 mg leucine per kg bw (corresponding to 7-10.5 g leucine at 70 kg bw) not only showed an effect on plasma levels of Isoleucine (decrease by 45%) and valine (decrease by 36%) but also on their levels in the cerebrospinal fluid (decrease by 53% in isoleucine, by 61% in valine) 2 hours after administration (Plaitakis et al., 1983).

With long-term daily doses of 45 mg leucine per kg bw (corresponding to 3.15 g per day at 70 kg bw) given to healthy individuals over 6 weeks, no effects on isoleucine and valine levels were observed (Crowe et al., 2006). With 7.5 g leucine per day (3 daily doses of 2.5 g) over 3 months, a statistically significant reduction in the (fasting) valine level by approx. 25% was registered. No effects on fasting levels of the aromatic amino acids phenylalanine, tyrosine and tryptophan or of methionine were recorded (Verhoeven et al., 2009). Statistically significant reductions in valine (18%) and isoleucine (13%) were observed when the same amount was administered to older type II diabetics over 6 months. The sum of the plasma levels of essential and non-essential amino acids showed no relevant changes (Leenders, 2011).

In healthy individuals, the clinical relevance of the observed antagonism of leucine to the other two branched-chain amino acids or from leucine (and possibly also from valine and isoleucine) to the other amino acids mentioned (primarily aromatic amino acids) is unclear. This also applies to the relevance of possible amino acid imbalances. However, the findings suggest that the three branched-chain amino acids should not be used individually, but all three combined.

With regard to possible interactions with aromatic amino acids (phenylalanine, tyrosine and tryptophan), it is discussed that BCAA doses¹⁷ cause a competitive inhibition of the transport of the aromatic amino acids at the blood-brain barrier¹⁸ by changing the plasma ratio of BCAAs to aromatic amino acids and that they could thereby influence the serotonin or catecholamine synthesis in the brain, which are based on these amino acids (Fernstrom, 2005; Blomstrand, 2006). This could possibly have psychological or mental (positive or negative) effects.

Leucine: Increase in blood ammonia levels and effects on blood urea levels

With doses of 50, 150, 250, 500, 750, 1000 or 1250 mg leucine per kg bw (corresponding to 3.5-87.5 g leucine at 70 kg bw), each administered to young adults (n = 5; 20-35 years) in one day, divided into several subsets over 8 hours, ammonia blood levels were significantly higher at the end of the study day than the fasting blood levels at the beginning of the study day for intakes of ≥ 150 mg leucine per kg bw. For intakes of 500 mg per kg bw (corresponding to 35 g for 70 kg bw) or more, at the end of the study day, exceedance of the normal range for blood ammonia levels was observed (normal range indicated by authors: < 35 micromole (μmol) per litre (L)). With an intake of 500 mg per kg bw, an average level of about $43 \mu\text{mol/L}$ ¹⁹ was registered. With an intake of 250 mg per kg bw (next lower dose level) the normal range was not exceeded. Ammonia levels were back to normal on the next day. Urea levels rose slightly in this group with intakes of ≥ 250 mg per kg bw, but were within the normal range for all intake levels (Elango et al.,

¹⁷ For combined BCAA doses see also below; Leucine is usually the largest fraction of combined BCAA doses.

¹⁸ The branched-chain amino acids and the aromatic amino acids phenylalanine, tyrosine and tryptophan are transported by a shared transporter at the blood-brain barrier.

¹⁹ Information estimated from graphic, specific values are not available from the publication.

2012). In elderly persons ($n = 6$; 72.2 ± 3.5 years), who were given doses of 50, 150, 250, 350, 450, 550, 650 and 750 mg leucine per kg bw, it was observed that ammonia levels exceeded the specified normal range (normal range: $< 35 \mu\text{mol/L}$) for doses ≥ 550 mg per kg bw.

In addition, blood urea levels exceeded the specified normal range (2.9-7.5 mmol/L) for intakes of 450 mg leucine per kg bw and above. It should be noted that blood urea levels in these elderly persons were close to the upper limit of the normal range (6.9 ± 1.1 mmol/L) even with intakes of 50 mg leucine per kg bw (roughly corresponds to the recommended daily intake for leucine). A statistically significant difference only existed with an intake of 650 mg per kg bw (Rasmussen et al., 2016).

In both studies, the oxidation of L-[1- ^{13}C]-leucine to $^{13}\text{CO}_2$ rose with increasing leucine doses and reached a plateau from certain intake levels. By means of regression analysis, plateau formation was observed in young adults with an intake of 550 mg per kg bw per day (95th confidence interval 454-647 mg per kg bw per day) and in elderly people with an intake of 431 mg per kg bw per day (95th confidence interval 351-511 mg per kg bw per day). The authors interpreted these intakes as marking the upper limit for the leucine oxidation of the body (Elango et al., 2012; Rasmussen et al., 2016).

The reported leucine intakes in both studies represent the total daily leucine intakes.

In both studies, the leucine dose was administered on one day only. No scientific data are currently available on the effects of long-term leucine administration on blood ammonia levels. It should also be noted that different values exist²⁰ for the upper limit of the normal range of blood ammonia levels (i.e. also higher values), thus further complicating the assessment of the blood levels observed by Elango et al. and Rasmussen et al.. However, the respective laboratory-specific normal ranges are conventionally used as the basis for the health-related evaluation of measured values.

In the organism, ammonia (NH_3) is primarily formed by the endogenous breakdown of amino acids as well as by bacterial protein breakdown in the intestine (see below for the formation during intensive physical exertion). Elevated ammonia blood levels could result from increased NH_3 flooding due to the breakdown of leucine or BCAAs, which should be the case here and/or from insufficient NH_3 elimination in the urea cycle, e.g. with impaired liver function. In congenital metabolic disorders of the urea cycle or in liver diseases, greatly increased blood ammonia levels are regarded as a major cause of brain damage that can occur in these diseases (although other endogenously formed substances can also have toxic effects in liver diseases) (Häberle and Koch, 2004; Filipo and Butterworth, 2002). Apart from the normal ranges for ammonia levels (which can vary between individual laboratories), hardly any generally accepted ammonia levels can be identified which should not be exceeded in order to protect human health (as a rough guide, it should be mentioned that with congenital disorders of the urea cycle drug treatment is recommended in case of ammonia levels above $100 \mu\text{mol/L}$; in such patients it is recommended to keep the plasma levels within the normal range, but at least below $80 \mu\text{mol/L}$ (DGKJ guideline; 2018)).

The blood ammonia levels observed by Elango et al. and Rasmussen et al. that exceed the normal range (normal range: $< 35 \mu\text{mol/L}$) with single-day intakes of 500 mg or 550

²⁰ E.g. the clinical dictionary "Psyhyrembel Klinisches Wörterbuch online" mentions a normal range of $16\text{--}53 \mu\text{mol/L}$ for ammonia in blood (plasma); however, the respective laboratory-specific standard values are conventionally used as the basis for the health-related evaluation of measured values.

mg leucine per kg bw, respectively, should be classified as changes in a biochemical laboratory parameter. Adequate information about the effects of prolonged leucine or BCAAs administration on blood ammonia levels is currently not available (see also section 3.7.3.4). The clinical relevance of the above findings with regard to possible adverse effects of prolonged leucine or BCAAs²¹ intakes cannot currently be clearly assessed due to the lack of corresponding long-term investigations in humans (ammonia levels, neurological examinations). Therefore, higher leucine and BCAAs intakes should be avoided.

The increased urea levels observed in older people indicate increased urea formation as a result of increased ammonia formation. The Exceedance of the normal range in older people with intakes of 450 mg per kg bw per day and above can be interpreted as changes in a laboratory parameter.

Leucine: Gastrointestinal disorders, other adverse effects observed

In young adults (20-35 years), no complaints or undesirable effects due to treatment were observed with single doses of 50 to 500 mg leucine per kg bw per day, with some subjects complaining of tiredness at the end of a test day. At higher leucine intakes (750-1000 mg per kg bw per day, corresponding to 52.5-70 g at 70 kg bw per day), one subject complained of gastrointestinal discomfort that subsided after the end of the study day (Elango et al., 2012). For single doses administered to the elderly (72.2 ± 3.5 years), one in 6 complained of gastrointestinal discomfort when taking 550 mg per kg bw per day (38.5 g at 70 kg bw) and one person about general tiredness at the study days at the same dose (Rasmussen et al., 2016).

In one study with long-term leucine administration of 10 g per day over 12 weeks in the elderly (85 ± 8 years), it is reported that 7 out of 15 people did not tolerate the leucine dose without providing further details ("*...seven subjects did not tolerate the supplement...*") (Trabal et al., 2015).

Leucine: Further information from clinical trials in healthy subjects

Existing studies of long-term intakes of single isolated leucine (i. e. without concomitant intake of other BCAAs) in healthy subjects cover durations of 5-12 weeks and intakes of approximately 3 to 10 g daily, administered only to small study groups ($n = 6-20$) (Mero et al., 1997; Crowe et al., 2006; Verhoeven, 2009; Ispoglou, 2011; Trabal et al., 2015). Information on the occurrence of undesirable effects is only available from the studies by Crowe et al. (2006) and Trabal et al. (2015). (In the opinion of the BfR, it cannot be concluded that no undesirable effects occurred during the course of a study simply from the fact that publications do not contain any information on the occurrence of undesirable effects.) The study by Crowe et al. with leucine doses of about 3 g per day (45 mg leucine per kg bw) over 6 weeks provides the information that no undesirable effects were observed. In the study by Trabal et al. (2015) with doses of 10 g per day over 12 weeks to elderly people (85 ± 8 years), it is reported that 7 out of 15 persons did not tolerate the supplement (see above). No information is available on the type and severity of the intolerance. From one of the studies cited above, there is information indicating that when 4

²¹ BCAA intakes are cited in this context because they also encompass leucine intakes, and because the three branched-chain amino acids are deaminated by the same aminotransferase isoenzymes (although it is recognised that there may be different affinities).

g of leucine were administered per day for 12 weeks, all examined laboratory parameters were within normal ranges, however no detailed information on this is provided (Ispoglou, 2011).

A short 14-day study with middle-aged subjects (52 ± 1 years) provides general information indicating that when 180 mg leucine were administered per kg bw per day (= 12.6 g per day at 70 kg bw) no undesirable effects were associated with the study participation (English et al., 2016).

Leucine: Further information from clinical trials in subjects suffering from diseases

From one study with the administration of 7.5 g of leucine to diabetics ($n = 30$) over 6 months there is information that the amino acid was well tolerated and no gastrointestinal symptoms were reported. Changes in glucose control were similar in the verum and placebo groups. Parameters related to the assessment of renal function remained unchanged (serum creatinine levels, 24-hour creatinine clearance). No relevant changes in fasting leucine levels were observed; isoleucine and valine fasting plasma levels were reduced (see above) (Leenders et al., 2011).

Leucine: Summary of human studies

Overall, very few human studies are available that contain relevant information for the risk assessment of isolated leucine intake.

Interactions of leucine doses with plasma levels (i.e. statistically significant reductions in levels) of the two branched-chain amino acids isoleucine and valine were observed with single doses in one study with 2 g leucine and in several studies with around 10 g per day (Swendseid et al., 1965, Elango et al., 2012; Rasmussen et al., 2016). With repeated doses, reductions in the levels of valine and/or valine and isoleucine were observed when 7.5 g of leucine were administered per day (Verhoeven et al., 2009; Leenders et al., 2011). No significant changes were observed with repeated daily doses of 45 mg leucine per kg bw (3.15 g at 70 kg bw) (Crowe et al., 2006).

With single doses of leucine, undesirable increases in ammonia levels that exceeded the normal range (normal range: $< 35 \mu\text{mol per L}$) were observed in young adults from 500 mg per kg bw and in elderly people from 550 mg per kg bw (corresponding to 37.0 or 38.5 g at 70 kg bw) (Elango et al., 2012; Rasmussen et al., 2016). Due to the different leucine doses that were gradually increased in both studies (see above), these two studies resulted in a NOAEL of 250 mg per kg bw based on this endpoint (Elango et al., 2012; young people) and of 450 mg per kg bw (Rasmussen et al., 2016, elderly). In older people, the blood urea level was above the specified normal range (2.9-7.5 mmol per L) from intakes of 450 mg per kg bw. A NOAEL of 350 mg per kg bw can be identified with regard to this endpoint (Rasmussen et al., 2016). There is no relevant information on the effects of longer-term higher isolated leucine doses on blood ammonia levels or urea levels (the publication by Ispoglou et al. (2011) only contains the general statement that when 4 g leucine were administered per day, all laboratory parameters examined were within normal ranges).

Adverse gastrointestinal effects were reported with single doses of 550 mg per kg bw and 750-1000 mg per kg bw (38.5 and 52.5-70 g with 70 kg bw) (Elango et al., 2012; Rasmussen et al., 2016). It should be noted here that the doses were only administered on one day and also only to small groups ($n = 5$ or 6). The relevance and validity of the findings of Trabal et al. (2015) (Indication that 7 out of 15 elderly people (85 ± 8 years)

did not tolerate intakes of 10 g leucine) are questionable due to the lack of detailed information, the advanced age of the group of people examined and the singularity of the findings.

3.7.3.2 Isoleucine

Isoleucine: Effects on plasma levels of branched-chain and other amino acids

Intakes of 2 g of isoleucine showed no statistically significant effects on the levels of the other two branched-chain amino acids or on the methionine, phenylalanine / tyrosine and threonine levels (Swendseid et al., 1965).

Isoleucine: Gastrointestinal disorders and other observed adverse effects

Guttuso et al. (2008) administered 5 g isoleucine per day (n = 50) or placebo (n = 50) over a period of 12 weeks to postmenopausal women who suffered from hot flashes. In a second study phase, both groups were divided into 2 groups, which received either 5 g valine (n = 46) or 2.5 g valine plus 2.5 g isoleucine per day over 10 weeks (n = 40). Furthermore, all patients received a preparation with various water-soluble vitamins.

In phase 1, with the administration of 5 g of isoleucine per day, 5 women in the verum group terminated the study prematurely due to undesirable effects (herpes zoster and oedema, arthralgia, stroke, infection, nausea) compared to just one in the placebo group (high blood pressure). The authors point out that the person who had the stroke had previously had high blood pressure. The most common undesirable effect was nausea (4 people with isoleucine versus 2 with placebo), next to the occurrence of oedema (not uncommon in this group of persons; 3 persons with isoleucine versus 2 with placebo). The relevance of the somewhat increased incidence of nausea for the derivation of upper intake levels is questionable due to the high "background noise" of this undesirable effect, the small difference between the verum and placebo group and the presence of such a finding from only one study to date. However, the finding is to be interpreted as the first indication of a possible occurrence of such undesirable effects, which still requires further confirmation.

In phase 2, with 5 g of valine or 2.5 g of valine + 2.5 g of isoleucine per day, 2 women terminated the study prematurely due to muscle pain and oedema (one from each study group). As this study phase does not include a placebo group and only the dropouts are detailed without any information regarding the occurrence of undesirable effects, the data cannot be interpreted with regard to the occurrence of undesirable effects.

Isoleucine: More information from human studies

Information on isoleucine from one study with administration of single doses by gastric tube indicates that doses of 5 or 10 g were tolerated without the occurrence of undesirable effects (Ullrich et al., 2016).

Overall, hardly any human studies are available with administration of isolated isoleucine doses that can be used for a risk assessment of this amino acid.

3.7.3.3 Valine

Valine: Effects on plasma levels of branched-chain and other amino acids

No statistically significant effects on the leucine and isoleucine levels or on the methionine, phenylalanine / tyrosine and threonine levels were observed with the administration of 2 g valine (Swendseid et al., 1965). With single doses of 62.5 mg valine per kg bw (approx. 4.4 g at 70 kg bw), reduced leucine and isoleucine levels were recorded 3 hours after ingestion (Schauder et al., 1984). No information is available on the effects of prolonged isolated valine administration on the levels of the other two amino acids or on the levels of the other amino acids mentioned.

Valine: More information from human studies

From one study, there is information that single doses of 30 g of valine did not lead to increased nausea (no further details on other undesirable effects). In individuals with depression who were symptom-free under medication, some cases of slight deterioration in mood were observed when a single dose of 30 g valine was given (Williamson, 1995). To what extent interactions with the tryptophan or serotonin metabolism may play a role here (e.g. via an antagonism at the blood-brain barrier) is currently unclear.

The findings from the study by Guttuso et al. (2008) with intakes of 5 g valine or 2.5 g valine + 2.5 g isoleucine per day cannot be interpreted with regard to the occurrence of undesirable effects.

Overall, hardly any human studies are available with administration of isolated valine doses that can be used for a risk assessment of this amino acid.

3.7.3.4 BCAAs (combined administration of leucine, isoleucine and valine)

BCAAs: Effects on plasma levels of other amino acids

In the case of combined single doses of the three branched-chain amino acids in a total of 5 g, the plasma levels of the aromatic amino acids (phenylalanine, tyrosine, tryptophan) and of methionine were significantly reduced after 3 hours (Zhang et al., 2011). In another study, with single doses of 10, 30 or 60 g BCAAs (4 parts leucine, 3 parts isoleucine, 3 parts valine) marked dose-dependent reductions in tryptophan, phenylalanine and tyrosine plasma levels were observed 5 hours after ingestion (by 45-63% with 10 g BCAAs; 60-85% with 60 g BCAAs) (Gijssman et al., 2002). However, due to the six-hour period without food intake and because no placebo controls were carried out, it remains unclear to what extent the observed reductions can be attributed solely to the BCAA intake or to fasting as well. However, the dose-dependent reductions suggest effects due to the BCAA administration. The extent to which this applies to the lowest intake already (10 g BCAA), cannot be estimated due to the lack of a placebo control. It is also currently unclear to what extent the observed level reductions are to be attributed to the administration of leucine or to the combined administration of the three branched-chain amino acids.

The above facts relate to administration of single BCAA doses. The health relevance of the observed interaction with plasma levels of aromatic amino acids is currently unclear. There are currently no relevant studies on the effects of long-term BCAA intakes on plasma levels of aromatic amino acids and on the question of whether these are associated with clinically tangible undesirable effects. It is discussed that BCAA administrations via a change in the plasma ratio of the BCAAs to the aromatic amino acids (phenylalanine, tyrosine and tryptophan) brings about a competitive inhibition of the transport of the aromatic amino acids at the blood-brain barrier and thus could affect serotonin or catecholamine synthesis in the brain which is based on these amino acids (Fernstrom, 2005; Blomstrand, 2006). To what extent this is associated with positive or negative effects, possibly in a dose-dependent manner, is currently unclear.

BCAAs: Increase in blood ammonia levels

Inconsistent information is available regarding the combined administration of the three branched-chain amino acids and their effects on ammonia levels. In addition, information on this is currently only available from studies in which higher amounts of BCAAs have been used. Tandan et al. (1996) observed an increase in serum ammonia levels of twice or more in 2 out of 29 patients with amyotrophic lateral sclerosis (ALS) who were given 26.4 g BCAAs per day for 6 months (no increase indicated in placebo group; increase in one out of 30 patients in a parallel group receiving 4 g threonine + 160 mg vitamin B₆ per day). However, at the start of the study, 6 out of 32 persons in the placebo group, 10 out of 31 in the BCAA group and 8 out of 32 in the threonine/vitamin B₆ group had elevated ammonia levels. This and the lack of detailed information on the extent of the individual ammonia levels limit the informative value of this study. In another study performed with ALS patients (n = 11) and with the same intake (26.4 g BCAAs or 12 g leucine, 8 g isoleucine, 6.4 g valine per day, respectively, divided into 4 equal doses) over 12 months, no significant differences in plasma ammonia levels between placebo (n = 11) and the small intervention group were observed. The informative value of these findings is limited because during the course of the study no attention was paid to the timespan between BCAAs intakes and blood sampling and/or the finding in individual persons could be blurred through the group comparison since corresponding detailed information is not available. However, the authors stated that in examinations at the start of the study, temporarily elevated ammonia levels were recorded one and two hours after oral BCAAs administration (Plaitakis et al., 1988). In a publication by Scarna et al. (2003), results from another study by the same author were cited, according to which increased plasma ammonia levels were observed over a period of 6 hours after administration of 60 g BCAAs (average maximum increase: 52 µmol/L; the authors consider plasma levels of ≥ 80 µmol/L to be critical to health).

Overall, there are first indications that in occasional cases significant increases in ammonia levels could occur with daily intakes of 26 g BCAAs over several days. However, there is considerable scientific uncertainty in this regard due to the scarce information available and inconsistent findings that are limited in their scientific significance. Information on increases in ammonia levels after BCAAs administration has so far only been available from studies with persons who suffered from amyotrophic lateral sclerosis; however, the increases observed there are in line with the findings after higher doses of leucine in otherwise healthy people (Elango et al., 2012; Rasmussen et al., 2016). Due to the lack of data, it is currently unclear whether and to what extent relevant increases in ammonia levels can occur with intakes below the BCAAs intakes mentioned above.

Intensive physical exertion is associated with (temporary) increases in blood ammonia levels. In these cases, in addition to the metabolic processes mentioned above (chapter

3.7.3.1), ammonia is formed by deamination of adenosine monophosphate (Yuan and Chan, 2000). In various studies, synergistic effects on ammonia blood levels were observed with single doses of about 5-23 g BCAAs and intense physical exertion, i.e. the temporary increases in plasma ammonia levels due to sport activities were increased by BCAA administration (e.g. after administration of 7.8 g BCAA and about 2 hours intensive cycling to 157 $\mu\text{mol/L}$ compared to 105 $\mu\text{mol/L}$ with cycling and placebo administration; van Hall et al., 1995) (van Hall et al., 1995; MacLean and Graham, 1993; Madsen et al., 1996). However, the health relevance of these sports-specific findings is currently unclear.

BCAA: Gastrointestinal disorders, other adverse effects observed

Administration of 10, 30 and 60 g BCAA (4 parts leucine, 3 parts isoleucine, 3 parts valine) to healthy persons ($n = 12$) was well tolerated according to the authors. With a dose of 30 g BCAA, one subject experienced mild gastrointestinal disorder 12-36 hours after ingestion, which, according to the authors, were probably not due to the amino acid intake (Gijsman et al., 2002). At high doses of 60 g BCAA per day (24 g leucine, 18 g isoleucine, 18 g valine) for 7 days in patients suffering from mania ($n = 13$), one patient each terminated the study prematurely due to nausea or tiredness, respectively (Scarna et al., 2003). In elderly people who were on dialysis and in a malnourished condition, transient diarrhoea was observed on 2 days in one out of 28 people with 12 g of BCAAs over 6 months (Hiroshige et al., 2001).

BCAAs: Further information from clinical trials in healthy subjects

In the case of combined administration of the three branched-chain amino acids to healthy adults, studies with a longer duration only include study periods of 14 to 63 days. The supplied intakes were mostly between 10-15 g BCAAs per day and ranged from 6 g per day up to 20 g per day (BCAAs administration over 42 days) or approx. 63 g per day²² (BCAAs administration over 19 days) in one study. The study groups were small with group sizes of ≤ 12 , whereby one study with 6 g BCAAs doses per day included 24 subjects (Sчена et al., 1992; Freyssenet et al., 1996; Mourier et al., 1997; Bassit et al., 2000; 2002; Coombes and McNaughton, 2000²³; De Palo et al., 2001; De Lorenzo et al., 2003; Tang, 2006; Trappe et al., 2007; Ra et al., 2013; Dudgeon et al., 2016). Overall, none of these studies provide relevant information on the occurrence of undesirable effects or on safety-related laboratory tests²⁴.

BCAAs: Further information from clinical trials in subjects suffering from diseases

No information on the occurrence of undesirable effects is available from a study involving intake of 10 g of BCAAs per day for 3 months by subjects suffering from heart failure (Pineda-Juarez et al., 2016).

When the branched-chain amino acids were administered at 26.4 g of BCAAs per day (12 g leucine, 8 g isoleucine, 6.4 g valine) for 6 months to patients with amyotrophic lateral sclerosis (ALS), it was indicated that the amino acids were well tolerated. The fre-

²² 0.9 g BCAAs per kg bw, corresponding to 63 g BCAA at 70 kg bw.

²³ In this study, 12 g of BCAA were administered daily, but on the 7th study day additional BCAA doses with a total of 52 g BCAA per day were administered.

²⁴ The information from the study by Schena et al. (1992) on BCAA intake during an Andean trekking tour cannot really be interpreted due to the limited information (only relating to undesirable gastrointestinal effects) and the relatively frequent occurrence of acute altitude sickness with nausea and vomiting.

quency of non-specific adverse effects (headache, gastrointestinal upset, anorexia, abdominal pain, constipation, insomnia, etc.) was higher in the placebo group (5 patients out of 32) than with BCAAs administration (2 patients out of 31). One patient in the BCAA group terminated the study prematurely due to the occurrence of gout. Observed effects on the ammonia blood level have already been described above. It is reported that no other adverse effects on haematological or biochemical parameters were observed, but no detailed information is provided in this regard. However, the study raised questions regarding a possible functional deterioration of the disease state of this patient group when BCAA was administered (Tandan et al., 1996). Another study with ALS patients ($n_{\text{BCAAs}} = 61$) with a similar dose (24 g of BCAAs per day or, 12 g leucine, 6 g isoleucine, 6 g valine per day, respectively) and planned duration of one year was terminated prematurely due to increased mortality in the BCAA group and no discernible benefit. In almost all cases, deterioration in respiratory function was the cause of death. Such deterioration is a known cause of mortality in ALS patients (*The Italian ALS Study Group*, 1993). These study results are therefore not transferable to healthy people.

There are several clinical studies that investigated combined doses of branched-chain amino acids in people with liver cirrhosis or other severe liver diseases, including among others, studies in which BCAAs doses were administered over longer periods of 1-4 years (e.g. Marchesini et al., 2003; Muto et al., 2005; Kobayashi et al., 2008; Habu et al., 2009; Yoshiji et al., 2012; Kawaguchi et al., 2014).

For example, Muto et al. (2005) reported that with administration of 12 g of BCAAs per day (5.71 g leucine, 2.85 g isoleucine, 3.43 g valine) to patients with liver cirrhosis over a median treatment period of 445 days in 38 of 317 patients undesirable effects were observed that mainly included gastrointestinal complaints such as abdominal distention, diarrhoea or constipation. However, no information is available on the occurrence of undesirable effects in the control group that received no additional BCAAs doses. The overall incidence of serious sequelae of liver diseases (deaths, rupture of varices, liver cancer, liver failure) was lower in the BCAAs group than in the placebo group. Another study reported that when 14.4 g of BCAAs per day (7.2 g leucine, 3.6 g isoleucine, 3.6 g valine) were administered to cirrhosis patients over 12 months, nausea, gastrointestinal discomfort, and diarrhoea were the most common reasons for "non-compliance", with no (statistically significant) difference between the verum (4 out of 59 patients) and the two control groups (2 out of 56 and 2 out of 59 patients) (Marchesini et al., 2003). Due to the severe metabolic disturbances associated with the underlying disease, the relevance of the undesirable effects observed in this patient group is questionable for the general population.

BCAAs: Summary of human studies

There are very few human studies that contain relevant information for the risk assessment of a combined intake of the three branched-chain amino acids.

With single doses of 5-60 g BCAAs, effects on the plasma levels of aromatic amino acids (phenylalanine, tyrosine, tryptophan) were observed (Zhang et al., 2011; Gijssman et al., 2002). The health relevance of the interaction with plasma levels of aromatic amino acids observed after single BCAAs doses is currently unclear. There is currently no relevant information available on the effects of long-term isolated BCAAs doses on plasma levels of aromatic amino acids or other amino acids and on the question of whether they have any undesirable clinical effects.

Twofold or higher increases in serum ammonia levels were recorded in a study with ALS patients in individual study participants with long-term doses of 26.4 g of BCAAs per day (Tandan et al., 1996). No information is currently available on the effects of lower BCAAs intakes on blood ammonia levels. It is noted that considerable scientific uncertainties regarding this undesirable effect (increases in the serum ammonia level) exist in relation to intake of BCAAs, due to the scarce information available and inconsistent findings that are limited in their scientific significance. However, the available findings provide indications on possible health risks associated with higher isolated BCAA intakes.

With doses of 60 g BCAA per day, the occurrence of nausea or tiredness was reported in one each out of 13 persons (Scarna et al., 2003). Adequate studies on the occurrence of nausea even with lower BCAA intakes are currently not available.

3.7.4 Children and adolescents

In children who suffered from Duchenne muscular dystrophy (median: 9.8 years) receiving 200 mg leucine per kg bw over 12 months, the most common undesirable effects were gastrointestinal effects, such as decreased appetite, anorexia, nausea and stomach discomfort, displaying equal incidences in the verum and placebo groups. According to the authors, laboratory analyses (blood count, BUN, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, total protein, blood sugar) showed no other deviations than those attributable to dystrophy. No detailed information is available on this (Mendell et al., 1984).

High doses of BCAAs were used in children and adolescents who suffered from phenylketone-uria (500 mg BCAAs per kg bw per day) or tardive dyskinesia (222 mg BCAAs per kg bw per day) (Berry et al., 1990; Richardson et al., 2004). However, due to the existing serious underlying diseases, these publications are not suitable for risk assessments of isolated BCAAs intake in children and adolescents.

Overall, there are no adequate studies in children and adolescents to assess the health risks of isolated intakes of branched-chain amino acids, individually or in combination.

3.7.5 Pregnant and breastfeeding women

Adequate studies to assess health risks of isolated intakes of branched-chain amino acids, as single BCAA or combination of BCAAs, in regard to pregnant and breastfeeding women could not be identified.

3.7.6 Congenital metabolic diseases

Maple syrup urine disease is a hereditary metabolic disorder affecting the breakdown of the branched-chain amino acids valine, leucine and isoleucine and is associated with very high BCAAs plasma levels, especially leucine plasma levels. It is associated with mental retardation and can even lead to premature death. However, information on this disease does not provide any useful data for assessing the health risks of oral BCAA intakes in healthy individuals (FNB, 2002/2005). The same applies to idiopathic leucine-induced hypoglycemia, a disease that is also associated with mental retardation and neurological disorders (Snyder and Robinson, 1967; Bicknell and Crome, 1969).

3.8 Risk characterisation

When assessing the health risks of isolated leucine, isoleucine and valine intakes, as single BCAA or in combination of BCAAs, considerable scientific uncertainties exist due to insufficient data. Adequate studies on the effects of prolonged, isolated intakes in healthy people are not available. Existing studies with healthy people include only a small number of subjects, usually only for a period of a few weeks, and often contain no meaningful information on the occurrence of undesirable effects. However, from the available scientific studies there are indications of possible health risks from higher intakes of branched-chain amino acids, as single BCAA or combination of BCAAs, which currently primarily concern undesirable changes in laboratory parameters (see below). Due to the insufficient scientific data, questions also arise as to whether and to what extent there are undetected health risks associated with high leucine, isoleucine, valine intakes, either as single BCAA or combination of BCAAs.

The following points are important for the risk assessment of the branched-chain amino acids:

3.8.1 Leucine

- In an animal study on sub-chronic toxicity (13 weeks), the highest intake level administered via the feed, i.e. 5 % leucine, was identified as the NOAEL, corresponding to an intake of 3.33 g per kg bw per day for male and 3.84 g per kg bw per day for female animals.
- Reductions in valine and isoleucine levels were observed with single doses of 2 g leucine. With doses given for several days, reductions in the valine and to a lesser extent the isoleucine fasting plasma levels were recorded when 7.5 g leucine was administered per day, whereas this was not the case with doses of approximately 3.2 g leucine per day (Swendseid et al., 1965; Verhoeven et al., 2009; Leenders et al., 2011, Crowe et al., 2006). The clinical relevance of the observed interactions cannot currently be conclusively assessed, but the interactions (as well as the interactions observed with valine administration) suggest that branched-chain amino acids should not be used as single BCAA but as combination of all three BCAAs²⁵.
- In two human studies, increases in blood ammonia levels beyond the normal range were observed with doses at 500 mg per kg bw (young adults) and 550 mg per kg bw (older people). For this endpoint, a NOAEL of 250 or 450 mg per kg bw (17.5 or 31.5 g at 70 kg bw) was identified from these studies due to the different incremental daily amounts of leucine administered.
In addition, in the elderly, the normal range of blood urea level was exceeded when the leucine dose was 450 mg/kg bw and above. A NOAEL of 350 mg per kg bw (24.5 g at 70 kg bw) was identified for this endpoint. It must be conceded that the subjects' urea levels were already close to the upper limit of the normal range before the start of the study. Nonetheless, the increase in this level and in particular the increase in

²⁵ The proportions of the three branched-chain amino acids can orient themselves by the ratios of the three amino acids that result from the guidance values derived for leucine, isoleucine and valine (see below) or from the recommended intake for leucine, isoleucine and valine derived by WHO (2007) or the American FNB (2002/2005).

the ammonia level illustrate an increased flooding of NH_3 as a result of the metabolism of leucine.

The clinical relevance of these findings, primarily the increase in ammonia levels beyond the normal range, is difficult to assess, especially since the leucine doses in both studies were only given over one day, and corresponding investigations in otherwise healthy subjects on the effects of higher leucine doses given over a longer period of time on ammonia levels are not available, nor are there investigations on clinical effects that may be associated with increased ammonia levels (e.g. of neurological nature). In addition, the findings were only collected from small groups ($n = 5$ or 6). In congenital metabolic disorders of the urea cycle or in liver diseases, greatly increased blood ammonia levels are regarded as a major cause of brain damage that can occur in these diseases (although in liver diseases other endogenously formed substances can also have toxic effects) (Häberle and Koch, 2004; Filipo and Butterworth, 2002). Apart from normal ranges for blood ammonia levels (which can vary between individual laboratories), there are hardly any generally accepted ammonia levels that should not be exceeded in the long term to protect human health. Overall, exceeding the normal range of blood ammonia is to be regarded as an undesirable health effect. Therefore, higher leucine intake should be avoided.

- Adverse gastrointestinal effects were reported by one in six subjects with single doses of 550 mg leucine per kg bw and by one in five subjects with single doses of 750-1000 mg per kg bw (38.5 and 52.5-70 g with 70 kg bw) (Elango et al., 2012; Rasmussen et al., 2016). A NOAEL of 450 mg per kg bw (31.5 g at 70 kg bw) was identified for this endpoint in both studies. However, the scientific uncertainties that result from the fact that the leucine was given only on one day and not over several days and also only to small study groups ($n = 5$ or 6) must be taken into account.

Overall, the following conclusions can be drawn from the facts presented:

Due to insufficient scientific data and large gaps in knowledge, there are considerable scientific uncertainties in the risk assessment of isolated leucine intakes. From the incomplete scientific data available, there are indications of possible health risks from isolated leucine intakes. Therefore, higher leucine intake should be avoided. However, the data currently available are insufficient to derive the lowest amount of leucine added to foods (indicated as amount per daily portion)), which, if exceeded, is likely to have harmful health effects. The observed exceedance of ammonia blood levels after single leucine doses cannot yet be ascribed the quality of a clinically-relevant harmful health effect. On the other hand, there are no corresponding long-term studies on the effects of higher leucine intakes on ammonia levels and on clinical (neurological) effects that could be associated with increased ammonia levels. Further, there is an overall lack of intervention studies involving longer-term leucine administration in which the occurrence of undesirable effects was adequately investigated and the results in this regard adequately reported.

- In view of the available indications on possible health risks of higher leucine intakes in isolated form, it seems advisable to derive health-based guidance values. The BfR has therefore derived guidance values for tolerable supplemental (additional) daily intakes of isolated leucine, i.e. daily intakes of isolated leucine, which can be ingested in addition to the leucine intake via the normal diet and which, based on the current state of knowledge, are unlikely to pose any risk or may pose only a very low risk of undesirable effects.

The above-mentioned undesirable effects observed in human studies (exceedance of the normal range of blood ammonia levels, undesirable gastrointestinal effects, interactions with other branched-chain amino acids), as well as other available human studies with leucine, are not suitable for the purpose of deriving such guidance values (among other things because of a lack of long-term studies regarding the above-mentioned undesirable effects, small study groups, open questions about the biological relevance of observed effects with single doses, the lack of information on the occurrence of undesirable effects and the lack of detailed information on laboratory findings in available clinical studies). In view of these circumstances - and this consideration also applies to isoleucine and valine - the available animal studies on the sub-chronic and chronic toxicity of these branched-chain amino acids, taking into account adequate safety factors, currently offer the best scientific basis for deriving a guidance value for tolerable supplemental daily intakes of isolated leucine.

In animal studies on sub-chronic toxicity (13 weeks) a NOAEL of 3.33 g per kg bw per day for male animals was identified for leucine. When deriving a guidance value for the tolerable supplemental daily intake for adults from the NOAEL identified in this study, default values recommended by EFSA (2012) are used largely, i.e.

- i) an uncertainty factor of 2 for the extrapolation from sub-chronic to chronic exposure (an additional uncertainty factor for only sub-chronic exposure appears justified, given the findings for isoleucine, where adverse effects were observed during chronic exposure that were not apparent during sub-chronic exposure),
- ii) an uncertainty factor of 10 to take into account interspecies variability,
- iii) an uncertainty factor of 3.16, rounded down to 3, to take into account interindividual variability (given that these amino acids are essential nutrients, a reduced uncertainty factor seems justified; otherwise normal default value = 10),
- iv) Adult body weight: 70 kg.

Taking these default values into account, an orientation value for a tolerable supplemental daily intake of isolated leucine of 3885 mg leucine²⁶, rounded to 4 g leucine per day can be derived for adults from the above NOAEL (3330 mg per kg bw).

3.8.2 Isoleucine

- In animal studies on chronic toxicity (104 weeks), a NOAEL of 925 mg per kg bw was derived.
- There are hardly any human studies available that can be used for the risk assessment of isolated isoleucine doses. In one study with 5 g isoleucine per day, a slight increase in the incidence of nausea compared to the placebo group was observed. However, the relevance of this finding is questionable. In animal studies on chronic toxicity, testicular damage was observed with high isoleucine intake (1788 mg per kg bw). No corresponding investigations or information are available on this toxicological endpoint from human studies with isolated isoleucine doses. Overall, due to a lack of data, there are considerable scientific uncertainties in the risk assessment of isolated isoleucine doses.

²⁶ Orientation value for the tolerable supplemental daily intake of isolated leucine = (3330 mg/kg bw per day: (2 x 10 x 3) x 70 kg = 3885 mg/day (at 70 kg bw)).

- The data currently available are insufficient to derive the lowest amount of isolated isoleucine added to foods (indicated amount per daily portion), which, if exceeded, is likely to have harmful health effects.
However, the available scientific data indicate potential health risks. Due to the existing uncertainties in the risk assessment of isolated isoleucine intakes, it seems appropriate to derive health-based guidance values for tolerable supplemental daily intakes of isolated isoleucine and to proceed in the same way as for leucine.

Based on the above NOAEL identified in animal studies, a guidance value for the tolerable supplemental daily intake of isolated isoleucine²⁷ of 2158 mg, rounded to 2.2 g isoleucine/day is derived (uncertainty factor of 10 to take interspecies variability into account; uncertainty factor of 3 to take into account inter-individual variability; uncertainty factor for extrapolation to chronic exposure does not apply, since the NOAEL was identified from an animal study on chronic toxicity).

3.8.3 Valine

- In animal studies on sub-chronic toxicity (13 weeks) a NOAEL of 1.85 g per kg bw per day for female and 1.60 g per kg bw for male animals was identified.
- There are hardly any human studies available that can be used for the risk assessment of isolated valine doses. Interactions with plasma levels of leucine and isoleucine were observed with the intake of 62.5 mg valine per kg bw (approx. 4.4 g at 70 kg bw). The relevance of these findings cannot be conclusively assessed, but they suggest that branched-chain amino acids should not be used individually but rather in combination of all BCAAs.

Overall, there is considerable uncertainty in the risk assessment of isolated valine intakes, due to insufficient scientific data in many areas.

- The data currently available are insufficient to derive the lowest amount of isolated valine added to foods (indicated as amount per daily portion), which, if exceeded, is likely to have harmful health effects.
Due to the existing considerable uncertainties in the health assessment of isolated valine intakes, it seems appropriate to derive health-based guidance values for tolerable supplemental daily intakes of isolated valine and to proceed in the same way as for leucine.

Based on the above NOAEL identified in animal studies, a guidance value for the tolerable supplemental daily intake of isolated valine²⁸ of 1,866 mg, rounded to 2.0 g valine per day for adults, is derived.

3.8.4 BCAAs (combined administration of leucine, isoleucine and valine)

- Adequate animal studies on the sub-chronic or chronic toxicity of combined doses of the three branched-chain amino acids could not be identified.

²⁷ Orientation value for the tolerable additional daily intake of isolated isoleucine = (925 mg/kg bw per day: (10 x 3) x 70 kg = 2158 mg/day (at 70 kg bw)).

²⁸ Orientation value for the tolerable additional daily intake of isolated Valine = (1600 mg/kg bw per day: (2 x 10 x 3) x 70 kg = 1866 mg/day (at 70 kg bw)).

- With single doses of 5-60 g BCAAs in human studies, effects on the plasma levels of aromatic amino acids (phenylalanine, tyrosine, tryptophan) were observed (Zhang et al., 2011; Gijssman et al., 2002). The health relevance of the interaction with plasma levels of aromatic amino acids observed after single doses of combined BCAAs is currently unclear. There is currently no relevant information available on the effects of long-term doses of combined isolated BCAAs on plasma levels of aromatic amino acids or other amino acids, or on the question of whether they are associated with any undesirable clinical effects.

Twofold or higher increases in serum ammonia levels were recorded in a study with ALS patients in individual study participants with long-term doses of 26.4 g BCAAs per day (Tandan et al., 1996). Due to the lack of data, it is currently unclear whether and to what extent relevant increases in ammonia levels can occur with intakes of combined BCAAs below this. With BCAA doses, there is considerable scientific uncertainty regarding the occurrence of this undesirable effect, due to scarce information or due to a lack of detailed information, inconsistent and in their scientific significance limited findings, and the availability of corresponding information only for ALS patients (although NH₃ increases were also observed following intake of high leucine doses in healthy people). It must also be conceded that the clinical relevance of such increases cannot currently be assessed conclusively. Based on the available findings, higher intakes of combined isolated BCAAs should nevertheless be avoided as long as there are no adequate human studies in which safety-relevant questions regarding increases of ammonia levels as a result of higher intakes of combined BCAAs have been investigated.

With doses of 60 g BCAAs per day, the occurrence of nausea or tiredness was reported in one each out of 13 persons (Scarna et al., 2003). Adequate studies on the question to what extent the occurrence of nausea may be observed even with lower intakes of combined BCAAs, are currently not available.

Overall, due to insufficient scientific data, there are considerable uncertainties in the risk assessment of isolated BCAA intakes.

- With regard to the isolated, combined addition of the branched-chain amino acids leucine, isoleucine and valine in foods, the currently available scientific data are not sufficient to derive the lowest amount of these three amino acids (indicated as amount per daily portion), which, if exceeded, is likely to have harmful health effects. However, the available scientific data from human studies indicate potential health risks. Due to the existing scientific uncertainties, it seems appropriate to derive health-based guidance values for the tolerable supplemental daily combined intake of the branched-chain amino acids. The available human studies with BCAA doses are not sufficient to derive an appropriate guidance value and corresponding animal studies on the sub-chronic or chronic toxicity of intakes of combined BCAAs are not available. To derive the guidance value for tolerable supplemental daily intakes of isolated BCAAs, the sum of the guidance values derived for the individual amino acids is used, which corresponds to 8.2 g BCAAs per day (4.0 g leucine, 2.2 g isoleucine and 2.0 g valine).

3.8.5 Special groups of people

- Children, adolescents, pregnant and breastfeeding women

Adequate studies on the risk assessment of isolated leucine, isoleucine, valine or combined BCAA intakes in pregnant women and breastfeeding women as well as in children and adolescents are currently not available. These population groups should therefore avoid isolated leucine, isoleucine, valine or combined BCAA intakes. According to the *Scientific Committee of the Spanish Agency for Food Safety and Nutrition*, food supplements with additions of isolated BCAAs should not be consumed by pregnant women and children. The *Health Council of the Netherlands* (1999) advises pregnant and breastfeeding women as well as children under the age of 13 against consuming isolated amino acids.

- Individuals with reduced kidney function

Due to general theoretical considerations and in view of the importance of the kidney in the excretion of amino acid metabolites, individuals with impaired kidney function should use relevant intakes of isolated amino acids only after consultation with a physician.

- Individuals who follow a low protein diet

Based primarily on general theoretical considerations, individuals on a low protein diet are more likely than the general population to experience amino acid imbalances as results of intakes of large amounts of isolated amino acids, due to the reduced protein intake. It is therefore recommended that this population group consult their physician before consuming products that contain relevant amounts of isolated leucine, isoleucine, valine or combined BCAAs.

Further information on amino acids and food supplements is available from the BfR website

Health assessment of amino acids (in German):

https://www.bfr.bund.de/de/gesundheitliche_bewertung_von_aminosaeuern-54420.html

Frequently Asked Questions on Food Supplements:

https://www.bfr.bund.de/en/frequently_asked_questions_on_food_supplements-70347.html

Food safety: Food supplements in sports (in German):

<https://www.bfr.bund.de/cm/350/nahrungsergaenzungsmittel-im-sport.pdf>



BfR "Opinions app"

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