The Director General

Maisons-Alfort, 26 May 2011

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the assessment of the risks associated with substances with nutritional or physiological effects with a view to restricting or prohibiting their use in foodstuffs

ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

1. REVIEW OF THE REQUEST

On 11 September 2007, the French Food Safety Agency (AFSSA) received a formal request from the Directorate General for Health (DGS), the Directorate General for Food (DGAL) and the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) for an assessment of substances with nutritional or physiological effects whose use in foodstuffs should be restricted or prohibited.

This Opinion has been structured as follows:

2. BACKGROUND

The referral falls within the scope of European Regulation (EC) No. 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods. Although covered by the same Regulation, the regulatory approach for vitamins and minerals differs to that for other substances. Specifically, for vitamins and minerals the Regulation provides for a positive list, whereas a negative list approach is used for other substances with nutritional or physiological effects. Vitamins and minerals were addressed in a previous formal request (2007-SA-0315).

The Regulation, in Chapter I, Article 2 defines "other substance" as "a substance other than a vitamin or mineral that has a nutritional or physiological effect".
The term "certain other substances" may therefore be supposed to include "amino acids, essential fatty acids, fibre and other carbohydrates, various plants and herbal extracts" as mentioned in Recital 1 of the Regulation.

The provisions relating to the addition of "other substances" to foods (Chapter III, Article 8) are applied if "a substance other than vitamins or minerals, or an ingredient containing a substance other than vitamins or minerals, is added to foods or used in the manufacture of foods under conditions that would result in the ingestion of amounts of this substance greatly exceeding those reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet and/or would otherwise present a potential risk to consumers".
On its own initiative or on the basis of information provided by the Member States, the Commission may decide, after an assessment of the available information in each case by the Authority and in accordance with the procedure referred to in paragraph 2 of Article 14, to include, if necessary, the substance or ingredient in Annex III. In particular:
a) if a harmful effect on health has been identified, the substance and/or ingredient containing the substance shall:

i) be placed in Annex III, Part A, and its addition to foods or its use in the manufacture of foods shall then be prohibited; or
ii) be placed in Annex III, Part B, and its addition to foods or its use in the manufacture of foods shall only be allowed under the conditions specified therein;

b) if the possibility of harmful effects on health is identified but scientific uncertainty persists, the substance shall be placed in Annex III, Part C.

In this context, the joint request from the DGCCRF, DGAL and DGS specifically addresses the following points:
"Initially, AFSSA[1] is being requested to prepare a summary of:

- *opinions it has produced on the addition to food of substances with nutritional or physiological effects. [...] This is the case, for example, for coenzyme Q10 and lycopene.*
- *more general deliberations that the Agency has held on certain groups of substances (phytoestrogens, conjugated linoleic acid, etc.) which in some cases have led to proposed restrictions or bans on the use of these groups of substances.*

"In both cases, these opinions should be updated as necessary in light of any more recent scientific data. In any event, the AFSSA recommendations can then be used to propose the inclusion of the identified substances in Annexes III-A and III-B of Regulation (EC) No 1925/2006.

"Subsequently, AFSSA may propose a supplementary list of substances for which possible harmful effects have been identified and which should undergo a risk assessment as a matter of priority. The scientific evidence implying the existence of some risk should be clearly stated. The Agency's recommendations can then be forwarded to EU level to request the inclusion of these substances in Annex III-C of Regulation (EC) No 1925/2006.

"Finally, the Regulation is based on quantities of substances "reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet". This notion, which is not clearly defined, merits further study if the French authorities wish to fully exploit the Regulation's provisions."

[1] ANSES was founded on 1st July 2010 following the merger of two French health agencies, the French Food Safety Agency (AFSSA) and the French Agency for Environmental and Occupational Health Safety (AFSSET)

3. EXPERT APPRAISAL METHOD

The collective expert appraisal was conducted by the Expert Committee (CES) on Human Nutrition which met on 20 November 2008, 23 April 2009 and 30 September 2010, and was based on the preliminary work of a sub-group of experts from this CES.

3.1. Scope of the expert appraisal

Given the information presented in the regulations, the following could potentially be included under the term "certain other substances":

- macro- and micronutrients other than vitamins and minerals, namely amino acids, essential fatty acids, fibre and other carbohydrates;
- chemically defined substances;
- probiotics;
- plants, algae, fungi and their extracts and foods containing them.

Thus, the category of "certain other substances" encompasses a heterogeneous group of substances, both in terms of their nature and of the scientific knowledge available.

AFSSA had issued several opinions on:

- the risks associated with the consumption of plants and their extracts: report entitled “Framework for the evaluation of the safety, the effect and the claims of foodstuffs, made from plants, for the human diet” (AFSSA 2003b); Opinion of 21 December 2007 (AFSSA 2007d) on the assessment of a draft decree on the use of nutritional and physiological substances and botanicals and botanical preparations in the manufacture of food supplements; Opinion of 14 December 2007 (AFSSA 2007c) on the use of botanical food supplements which have been the subject of drug safety monitoring reports;
- the risks associated with the use of plants, algae and fungi, and preparations of plants, algae and fungi in the manufacture of food supplements (AFSSA 2007d; AFSSA 2008c; AFSSA 2008d).

ANSES considers that the findings of these opinions are applicable to the assessment of the risks associated with the consumption of the substances mentioned in all foods, including food supplements.
In addition, EFSA deliberated, in conjunction with the Member States, on the use of plants in human foods (ESCO[2] Working Group on Botanicals) (EFSA 2009a).
Consequently, ANSES will consider in this Opinion only those substances included under the term "other substances" which exclude plants, algae, fungi, and their extracts and foods containing them.

Moreover, ANSES considers that the deliberation should take into account all the foods likely to contribute "certain other substances" in addition to those which are specifically fortified. Therefore, the presence of these substances in food supplements will also be considered, in exactly the same way as the approach adopted at national and EU levels for fortification with vitamins and minerals.

3.2. Working method

The ANSES expert appraisal was conducted in two stages. Initially, ANSES compiled a list of the opinions and reports published on some of the "other substances" **(Annex 1).** In most cases, these earlier assessments concerned industrial dossiers related to products with clearly defined compositions, consumption levels, claims and evidence. Other cases related to questions of a general nature about substances or ingredients containing them (phytoestrogens, lycopene, linseed oil, etc.).
In the opinions relating to industrial dossiers, information on risk was not always stated and when it was, it related to the end product and not the substance.

[2] EFSA scientific cooperation

For this reason, all these opinions cannot be used as a basis for risk assessment in the context of this request. A full risk assessment was therefore necessary.

Subsequently, and from this initial list of substances, ANSES selected, as an example, four groups of substances: phytoestrogens, conjugated linoleic acids (CLA), fibre and other carbohydrates, and amino acids.
This choice was motivated by the availability of information relating to risk, mentioned in the literature, or any other warning data. In addition, these groups of substances reflect market reality. Evaluating them illustrates the difficulties associated with the assessment of risks relating to fortification in "other substances".

Where substances had been addressed as part of previous general deliberations, the assessment consisted of: 1) an update of the data when a risk assessment had previously been conducted (CLA, phytoestrogens), 2) a full risk assessment when only the benefits had been considered (fibre).

The safety of use in foods, including food supplements, of 31 substances was recently assessed (AFSSA 2008b).
ANSES reiterates that for each substance included in this Opinion, the proposed assessment should not be considered as exhaustive. The aim is to illustrate, through examples, the risks associated with the process of fortification. Therefore, the absence of any reference to a substance does not mean that it is unlikely to pose a risk to the consumer when added to a food.

4. DISCUSSION

The rationale of the French Agency for Food, Environmental and Occupational Health & Safety is based on the opinion of the Expert Committee on Human Nutrition whose points are presented below:

4.1. General introduction to the fortification of foods in nutrients or substances

4.1.1. General considerations on fortification

The analytical information presented in this section applies equally to "other substances" and to vitamins and minerals.

❖ **Nutritional and physiological effects**

According to the Regulation, the nutrient or substance involved in the fortification must have a nutritional or physiological effect. However, the definition of the nutritional or physiological effect is not specified. In its report entitled "Framework for the evaluation of the safety, effect and the claims of foodstuffs, made from plants, for the human diet", AFSSA stressed that "nutrition cannot be dissociated from physiology. Nutrients act in accordance with an energy-related and/or a non-energy related aspect in structural terms and on the various systems of the body's physiology" (AFSSA 2003b).
In the remainder of this document, these effects will therefore be identified as "nutritional/physiological effects".

ANSES proposes taking the following into account:

- For vitamins and minerals and "other substances", for which there are reference values (such as population reference intakes (ANCs) in France or Dietary Reference Values (DRVs) at European level), there are data showing that at these reference intake levels, these nutrients do indeed have a nutritional/physiological effect. As a result, the nutritional/physiological effect can only be observed in the event of inadequate intake in the population or in a population sub-group (AFSSA 2004b). Thus, the fortification proposed by the public health authorities concerns nutrients for which an insufficient intake or a

deficiency is found in a part of the population. This might include for example vitamin D in milk and dairy products as well as iodine and fluorine in salt. In these cases, a specific study of the suitable food vectors is performed to ensure that fortification can provide better coverage of the needs of these populations while avoiding overexposure.

- For vitamins and minerals and "other substances" for which there are no reference values, ANSES considers that only the demonstration of a specific nutritional/physiological benefit covered by generic or specific claims authorised by the European Commission, following an opinion issued by EFSA, should allow their addition to food, subject to their safety of use being demonstrated.

❖ Risks associated with fortification

The risks associated with the addition of vitamins and minerals and "other substances" to foods can be of different types:

- *Intrinsic risks*

These are toxic risks specific to the substances, that can be analysed using toxicological methods, which implies the existence of data from experimental studies. Concerning nutrients and substances already found in foods and consumed for long periods at levels provided by normal diets, there are frequently no toxicological studies. It is therefore necessary in this case to take into account data from medium- and long-term epidemiological and clinical studies, which may call into question the benefit of fortification at intake levels considered toxicologically safe. For example, studies have questioned the safety of supplementation with beta-carotene (ATBC 1994; Omenn *et al.* 1996) or various antioxidants (Bjelakovic *et al.* 2008). More recently, it was suggested that, even with fortification defined by public health authorities, the risks are not zero. The case of vitamin B9 is a case in point. In some countries it was used to fortify a limited number of vectors (flour and other grain products) to a given level, defined by the public health authorities, to prevent neural tube closure defects. However, new data led to a reconsideration of the potential risks associated with this fortification relating to the development of some cancers (colorectal, prostate, breast) (EFSA 2009b). Consequently, extending fortification to an entire population based on results from limited studies is always a risk. It requires, in addition to a prior assessment, the implementation of monitoring tools.

- *Risks associated with changes in bioavailability and with the effects of food matrices*

These risks depend on the level of intake, as well as the bioavailability of the substance in the food. The latter can vary from one matrix to another, leading to very different kinetics of absorption and plasma concentrations, and thus potentially different biological effects. In the case of lycopene, for example, the bioavailability is higher for synthetic forms in an oil complex than for natural forms found in tomato puree extracts (AFSSA 2004a). New biotechnologies, including nanotechnology, can also affect forms of intake, and consequently biological exposure to substances (AFSSA 2009b; EFSA 2009c).
Moreover, the case of substances with no toxic effect threshold, such as those involved in immuno-allergic reactions, must be considered.
These changes in bioavailability and matrix effects can thus lead to a risk without the amounts used necessarily "greatly" exceeding "those reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet".

- *Risks associated with production processes (physico-chemical processing of the substance)*

The physico-chemical processes applied to foods can alter the physico-chemical properties of the substances added. Similarly, interactions can occur with other constituents of the foods. These changes, which may occur during industrial or domestic processes, can lead to the potential alteration of the substances involved. The example of *trans* fatty acids produced by different technological processes applied to fats can be mentioned in this regard (AFSSA 2005a).

- *Risks associated with secondary metabolites*

Metabolites from the substances added may have specific biological effects. For example, lycopene and beta-carotene have secondary metabolites, which may have effects comparable to those of retinol and retinoic acid (Opinion of 30 April 2008, Request No. 2007-SA-0306).

- *Risks associated with interactions between substances*

The addition of certain substances can affect the bioavailability of nutrients: this is the case with phytates that limit the absorption of calcium (Gueguen and Pointillart 2000) or theaflavins found in tea that limit the absorption of iron (Zijp *et al.* 2000). There may also be phenomena of competition between different substances, for example involving the same carriers or the same enzymes: this is the case with competition between omega 6 and omega 3 fatty acids with regard to desaturases (Legrand *et al.* 2001).

- *Risks associated with interactions between drugs and substances*

For example, hesperidin (found in citrus fruits in particular) is known to interact with many drugs involved in the treatment of various conditions such as anxiety, depression, hypertension and cancer (AFSSA 2008b).

- *Risks associated with specific characteristics of certain sub-populations*

Because of their physiological condition, some population groups may be more vulnerable than others. Thus, the characteristics of children, pregnant or breastfeeding women or the elderly have led to the definition of nutritional guidelines specific to each group.
Moreover, genetic polymorphism may have an effect, even if it is taken into account, at least partly, by the use of large-scale populations. For example, alpha-lipoic acid intake may cause autoimmune syndrome in genetically susceptible individuals (AFSSA 2008b). This point is taken into account, at least for the known risks, in the deliberation on the generic claims conducted by EFSA, whose opinion is particularly sought on population groups for whom consumption of the substance may present a risk. It should nevertheless be noted that where there is a low prevalence of a genetic variant in the population, it is unlikely that a more vulnerable sub-population can be easily identified. Finally, even if the physiological or genetic characteristics of the population at risk can be identified, the individuals with these characteristics or genotypes are generally unaware of them. For example, people with a gene for susceptibility to the immunotoxic effects of lipoic acid are unaware of it most of the time.

- *Risk of overexposure due to the increase in food sources*

In the current context of possible cumulative intake from the consumption of several food vectors (food supplements, fortified foods, non-fortified foods, additives, etc.), overexposure to substances should not be overlooked. In the INCA2 study, nearly 20% of adults declared that they had consumed at least one food supplement during the previous year and just over 11% during the seven days of the survey (AFSSA 2009a). These data suggest the existence of a non-negligible risk of overexposure.

- *Behavioural risks*

ANSES reiterates that apart from objectively recognised situations of inadequate intake, consumption levels of these "other substances" should be achieved from a varied and balanced diet and not from additional intakes. Using claims to promote the benefits of fortification can lead to changes in eating behaviour. As well as the more or less systematic selection of fortified products which can lead to overexposure as previously discussed, consumers could move away from foods that are natural vectors of these "other substances", which would lead to dietary and nutritional imbalances (Mariotti *et al.* 2010; Hasler 2008).

4.1.2. Fortification levels

The Regulation stipulates that the provisions relating to the addition of "other substances" to food be applied when these "other substances" are added in amounts "greatly exceeding those reasonably expected to be ingested under normal conditions of consumption of a balanced and varied diet".
It is therefore up to ANSES to estimate the level of intake of the "other substances" achieved by the normal diet.
In the case of substances for which consumption data exist, ANSES estimates that the amounts "reasonably expected to be ingested under normal conditions of consumption of a balanced and

varied diet" could be defined on the basis of intake levels of the highest consumers (90th percentile), provided that they are not likely to pose a risk to the consumer. This level of intake should take account of all food sources for the substance.
However, for many substances, the usual level of consumption is not known, because of the lack of data on food composition. Indeed, these are substances for which there are few or no data with which to assess their nutritional value (especially when a nutritional need has not been identified). This is the case for instance with astaxanthin, lycopene, alpha-lipoic acid or gamma-aminobutyric acid (GABA).

4.1.3. Specific characteristics of fortification in "other substances" compared to fortification in vitamins and minerals

The approach taken by AFSSA for fortification in vitamins and minerals was based on maximum consumption data and the safety limit, in order to facilitate the determination of a maximum fortification limit guaranteeing an acceptable level of safety. But regarding other substances, in most cases no safety limit has been defined and/or no consumption data are available.
Therefore, even in situations where physiological effects may have been demonstrated for a substance (as is the case with vitamins and minerals), merely knowing the effective levels of intake is not sufficient to justify the safety of fortification.

- **Lack of a safety limit**

For substances that were assessed as part of the "novel foods" procedure (Regulation 258/97), data on safety were studied. However, these data are often lacking for the "other substances" already present in foods and not covered by this procedure.
Various approaches have been developed to overcome the lack of safety limits, including taking into account the highest amounts not leading to adverse effects in studies in humans. One example is the OSL (Observed Safe Level) method which requires, in practice, a series of studies and proceeds by examining each study, starting from the highest dose administered (Hathcock and Shao 2008). For each study not reporting adverse effects, and which could conventionally be used to set an NOAEL (No Observed Adverse Effect Level), it is necessary to determine whether the uncertainty is sufficiently low to enable an uncertainty factor of 1 to be applied. Iteratively, as the review progresses through decreasing doses in the series of studies, it arrives at a study (and dose) for which the uncertainty is low (good quality, many parameters studied, long duration, etc.). Applying an uncertainty factor of 1 for this study is then legitimised by the existence of other studies of lesser quality but conducted with higher doses and which did not report adverse effects.
The FAO/WHO proposed a similar approach, based on the HOI (Highest Observed Intake) applied in the absence of an identified hazard and administered or reported in studies of acceptable quality (FAO/WHO, 2006).
These approaches have many limitations. Indeed, supplementation studies in humans have been conducted almost exclusively in small cohorts and for short durations. They are therefore statistically ineffective for ruling out the risk in very large populations, which is the objective for public health. This is especially true since genetic and environmental characteristics vary widely in the general population. Moreover, the possibility of a slow induction of physiological disorders (e.g. induction of insulin resistance) is difficult to assess in the short or medium term. Last but not least, most of the studies considered were not designed to assess the presence of side effects, but rather the expected positive effects. In addition, they were not systematic in nature and only studied certain functions or organs and a few markers. Studies in humans should use as evaluation criteria the markers corresponding to the adverse effects identified by screening studies in animals (Renwick 2004).
Thus, in most cases, studies in humans can only be used to examine the tolerance of a substance and its acute toxicity, which are insufficient criteria with which to assess the safety of consumption in the medium and long term.

In addition, by limiting fortification to "other substances" for which a nutritional/physiological effect has been acknowledged, assessing the risk associated with the addition of this substance may be limited to conditions of use (doses and food vectors) defined for the purpose of claims. This

approach would be in accordance with the one recommended previously (AFSSA 2008b; AFSSA 2007b).

❖ **Insufficiency of intake data for "other substances"**

There are uncertainties and often gaps in the data on intakes of nutrients and substances. These relate to the composition data (missing data, inaccuracies in the existing data: e.g. amino acids, long-chain polyunsaturated fatty acids), the difficulties associated with assaying and changes in consumption data.

If despite all this, other considerations were to lead to the marketing of products fortified with "other substances", the consumer should be informed by appropriate labelling of the lack of a risk assessment. A similar approach is followed by the FDA as part of the evaluation of claims made for food supplements[3].

4.1.4. Aiming for a benefit/risk analysis

European regulations on the addition of nutrients and substances to foods are based on several texts relating to complementary and essential concepts. Accordingly, the benefits are assessed through a process (Regulation 1924/2006 on claims) that is independent of risk assessment (Regulations 258/97 and 1925/2006). Under these conditions, it is difficult to balance these two aspects.
As illustrated by the example of fortification of flour with vitamin B9, the risk may sometimes only be raised several years later, in line with the development of scientific data. Thus, the high level of control of fortification with a nutrient is not necessarily sufficient to limit the risk. In situations where the conditions of use (substance, food vector, quantity, etc.) have not been stipulated in advance, the risks are *a priori* greater.
For this reason, ANSES considers that in the absence of a proven benefit for a population, taking even a minimal risk is unjustified. Moreover, even in situations where the nutritional/physiological effects may be demonstrated for a substance, merely knowing the effective levels of intake is not sufficient to justify fortification. The risk associated with this potential beneficial effect should also be assessed. The question may arise whether, in certain specific situations of insufficient intake or even proven deficiency, making the nutrients concerned available to the target population as a concentrate (food supplement) or in very specific vectors would not be preferable to the fortification of common foods. The case of vegans could be mentioned, with regard to the coverage of their vitamin B12 needs. Part B of annex III of the Regulation, by proposing the restricted use of substances (under specified conditions of use) allows, in principle, this type of limitation.

4.2. Illustration of the limitations of the assessment of the risks associated with fortification in "other substances"

4.2.1. Case of amino acids

The considerations discussed in this Opinion relate to proteinogenic amino acids found in foods, whether or not essential. They exclude non-food amino acids and/or endogenous derivatives (e.g. 5-hydroxytryptophan, S-adenosyl-methionine, glutathione). Although these substances are marketed and some have been the subject of opinions from the Agency, little research has been conducted on their metabolism or to determine risk data. This limit to the scope of expertise also covers amino acid precursors (N-acetylcysteine, for example) for which there is some literature, but which are active ingredients in proprietary pharmaceutical products.

[3] http://www.fda.gov/Food/LabelingNutrition/LabelClaims/StructureFunctionClaims/default.htm
"Structure/function claims may also describe a benefit related to a nutrient deficiency disease (like vitamin C and scurvy), as long as the statement also tells how widespread such a disease is in the United States. The manufacturer is responsible for ensuring the accuracy and truthfulness of these claims; they are not pre-approved by FDA but must be truthful and not misleading. If a dietary supplement label includes such a claim, it must state in a "disclaimer" that FDA has not evaluated the claim. The disclaimer must also state that the dietary supplement product is not intended to "diagnose, treat, cure or prevent any disease," because only a drug can legally make such a claim."

❖ **Amino acid metabolism and risk of overconsumption**

The spontaneous protein intake of the general population (with the exception of specific sub-populations such as frail elderly people) is well above the recommended dietary intake (AFSSA 2008a). Any physiological effects, if they were to be scientifically acknowledged, therefore correspond to effects "beyond" the requirement defined by the nitrogen balance equilibrium. On a nutritional and metabolic level, consumption of an amino acid at levels much higher than other amino acids, and much higher than the corresponding quantitative requirements for protein synthesis, induces changes in circulating pools, changes in functions directly controlled by the amino acids, the substantial entry of these amino acids into catabolic pathways (sometimes in "secondary" metabolic pathways) and the activation of excretion pathways.
Thus, an amino acid intake that exceeds requirements induces metabolic exposure beyond the zone of homeostasis and the possibility of entering a toxic zone, as recognised by the scientific community (**Figure 1**). Therefore, even if the substance is a nutrient, the uncertainty factors used to calculate the upper limits should, *a priori,* be the same as those used to assess the risk associated with exposure to xenobiotics (Renwick 2006).

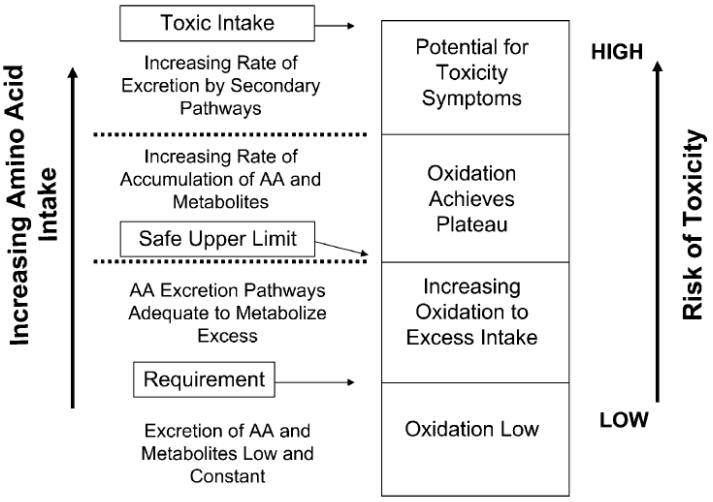

Figure 1. Schematic representation of body responses to excess amino acid (AA) intake. From Pencharz *et al.* (2008)

The level of intake of an amino acid inducing activation of its catabolism is the basis for establishing the requirement but also the potential toxicity range for this amino acid. Beyond this, a toxic risk is possible (Bier 2003), and it must be determined (1) whether the level of intake which leads (would lead) to a benefit is less than that at which toxicity appears, and (2) whether the level of toxic intake is likely to be reached in a fraction of the population, given the inter-individual variations, especially related to genetic polymorphisms. Several authors (Pencharz *et al.* 2008; Bier 2003) have suggested that the metabolic response to different levels of amino acid consumption can be used as a criterion for defining the maximum intake level. This involves defining the point at which the oxidation capacity reaches a plateau (Pencharz *et al.* 2008), or comparing the curve describing the oxidation as a function of the circulating concentration (or dietary intake) and that describing the benefits due to intake (**Figure 2**) (Bier 2003).
This approach is difficult for amino acids with many metabolic and catabolic pathways.

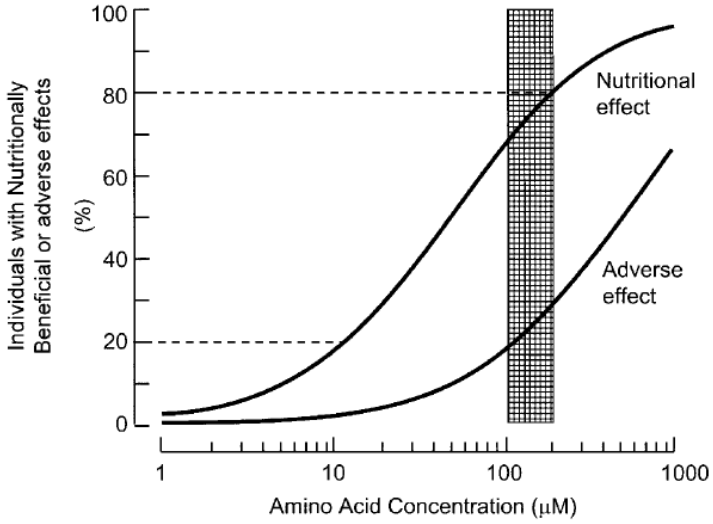

Figure 2. Hypothetical benefit/adverse effect curves as a function of circulating amino acid concentration (or ingested dose). The hatched area indicates a potentially safe intake range defined by maximal beneficial effects with correspondingly limited adverse consequences. From Bier *et al.* (2003).

❖ **Lack of sufficient data for characterising the hazards**

The example of amino acids is illustrative of the scarcity or absence of toxicological data on "other substances".
The established fields of toxicokinetics (the relationship between the dose of the product administered and the extent of the exposure at its site of action) and toxicodynamics (the relationship between the exposure of a toxin at its site of action and the subsequent functional effects) have not been applied to amino acid metabolism (Bier 2003). Only lysine and leucine have undergone fairly extensive kinetic modelling studies.
In cases where a hazard has been identified, the lack of these data prevents detailed uncertainty factors from being defined (Renwick 2004). Therefore, based on the available data, only an uncertainty factor of 100, typically used in toxicology, would be justified. However, the application of this uncertainty factor is often not very satisfactory, and it sometimes refers to a definition of the upper limit of toxicity which is lower than the requirement in amino acids, or lower than the adequate intake. This is the case with glycine, whose NOAEL is estimated at 1800 mg/kg of body weight; for a 60 kg man, the safety limit would be 1.1 g per day, whereas spontaneous intakes amount to several grams (Sakai *et al.* 2004).
Nevertheless, this finding concerning the overvaluation of the uncertainty factor of 100 should not lead to the rejection of the principle of calculating the upper limit on the basis of an NOAEL and an uncertainty factor: it draws attention to the crucial lack of data for characterising the dose-response kinetics in humans and animals, and their intra-species variability.
The OSL and HOI methods described above enable the use of the uncertainty factor to be refined objectively, so that an upper limit can be calculated fairly precisely once an NOAEL has been documented (FAO/WHO 2006).
The OSL method has been applied to different nutrients, including amino acids (Shao and Hathcock 2008).
On the contrary, the HOI method is only applied in the absence of an identified hazard, which is not the case with amino acids, since data reporting adverse effects can almost always be found.
However, these methods have several limitations (detailed above).
Therefore, in most cases, studies in humans can only be used with relative confidence to examine acute toxicity, fortunately lacking at the doses used, and tolerance, which is not sufficient in itself as a safety criterion.
Finally, as will be seen below, accurate data are needed to characterise the hazard. These studies cannot be conducted in humans, and require the deployment of traditional toxicological approaches, as with additives or contaminants.

❖ **The need for extensive toxicological studies**

Basic toxicological data are indispensable, especially in the case of amino acids (Renwick 2004). Screening for the potential hazards associated with the consumption of amino acids beyond spontaneous intake requires conventional animal studies. In the context of amino acids, very specific and thorough examinations are required depending on the metabolic pathways and physiological functions associated with each amino acid. For example, it is essential to have neurotoxicity data on amino acid precursors of neurotransmitters (e.g. tryptophan) (Renwick 2004).
Most amino acids have multiple, intricate metabolic pathways **(Table 1).** These may be linked to those of other amino acids, certain autacoids or other nutrients (vitamins). This may lead to the possibility of (1) competition or synergies between the metabolic pathways of different amino acids, (2) toxicity transported by a secondary metabolite intervening far later and often underestimated, (3) unexpected effects on functions, for which no robust hypothesis has yet been advanced (Garlick 2001).

Table 1. Some metabolic products and documented adverse effects of amino acids. From Garlick (2001). The studies considered examined the effects of amino acid supplementation.

Amino acid	Metabolism	Reported adverse effect
Alanine	Urea, lactate, glucose	Hyperammonemia
Arginine	Urea, ornithine, citrulline, NO, polyamines	Hypotension, tumor stimulation, acidosis, hyperkalemia, cardiac arrest
Aspartate	Urea, asparagine, glucose	Neurotoxicity
Cysteine	SO_4^{2-}, glutamate	Hypercholesterolemia, fatty liver, neurotoxicity
Glutamate	Urea, glutamine, glucose	Neurotoxicity, Chinese Restaurant Syndrome
Glycine	Serine, NH_4^+	Neurotoxicity, hypernatremia
Histidine	Histamine, formiminioglutamate, urocanate, glutamate	Hypercholesterolemia,
Isoleucine	Glutamine, alanine, ketomethylvalerate	Mental retardation
Leucine	Glutamine, alanine, ketoisocapriate	Hypoglycemia, mental retardation, hypovalinemia, hypoisoleucinemia
Lysine	Saccharopine, aminoadipate	Arginine antagonism, tubulointerstitial nephritis
Methionine	Cysteine, homocysteine, cystathionine	Hyperhomocysteinemia, serum folate deficiency
Phenylalanine	Tyrosine, phenylpyruvate	Mental retardation, exacerbates tardive dyskinesia in schizophrenics
Tryptophan	Serotonin, melatonin	Eosinophilia-myalgia syndrome, serotonin syndrome
Tyrosine	Tyramine, catecholamines, thyroid hormones, parahydroxyphenylpyruvate	Eye and skin lesions, mental retardation
Valine	Glutamine, alanine, ketoisovalerate	Mental retardation

In addition, these pathways may be sensitive to interactions with drugs or environmental variables (, diet, etc.). Finally, it is common for a major genetic polymorphism to considerably change a complex metabolic situation. This has often been shown. Where this is not the case, a more subtle polymorphism has often been suggested (see below for tryptophan).

- *Example of tryptophan*

Tryptophan metabolism is complex. In addition to the "tryptophan → serotonin" relationship, this metabolism has many ramifications which are sensitive to the nutrient status, particularly with regard to vitamins B6, B2 and B3 (Horwitt *et al.* 1981), to environmental interactions and to genetic polymorphism, especially on the major catabolic pathways of this amino acid (Oxenkrug 2007;

Raitala *et al.* 2005). This suggests a wide variety of metabolic and physiological responses to a supra-nutritional intake of tryptophan. Finally, there is the possibility of interaction with drugs (antidepressants) acting on the metabolism of tryptophan and serotonin.

- *Example of glutamine*

Another example of the multitude of potential hazards is provided by glutamine, which has quite good tolerance in humans. However, there are many targets, in terms of metabolism and organs, and many signalling pathways that may be affected (Watford 2008). In particular, there are interconversions between glutamine, glutamate, proline and ornithine/arginine (Watford 2008). These effects are expected to be silent at first, but can then reveal themselves in the medium to long term.
Finally, the complexity of glutamine metabolism, including the synthesis pathway of glutamate and ammonia, which both have neurological effects, implies the need for specific behavioural and psychological studies (Garlick 2001).

- *Other examples of amino acids*

The metabolism of sulphur amino acids (methionine, cysteine) is another example of metabolic entanglement, related to important biological intermediates (homocysteine) and connected to the crucial metabolism of labile methyl groups. Thus, some potential adverse effects of methionine supplementation have not been evaluated. These include immunological and inflammatory effects associated with supplementation in this amino acid, whose possibility was identified *in vitro,* but which is technically more difficult to assess *in vivo* (Grimble 2006). This case provides an example of a target for extensive toxicological studies in animals.

- *Beyond toxicological studies*

The metabolic and physiological complexity of most amino acids means that only broad-spectrum studies are able to demonstrate deleterious effects. Many authors argue the case for "-omics" ("systems biology") type approaches, especially nutrigenomics and metabolomics (Elliott *et al.* 2007; Morris 2007). In addition, the inter-individual variability from genetic polymorphism calls for nutrigenetics data, to assess the metabolic and hence toxicity differences between individuals (Hesketh *et al.* 2006).
Finally, there is a need for studies that are both long-term and sufficiently detailed in terms of evaluation criteria. Indeed, as mentioned above, it is necessary to be able to identify the silent effects from pathophysiological markers likely to correspond to subtle changes in risk over the long term. Moreover, beyond conventional toxicology studies, in view of the possible interference with environmental characteristics, including diet, the hazard should be identified and characterised in different situations likely to affect the onset of toxicity. For example, it was only recently that some authors showed that supplementation with branched side-chain amino acids increases resistance to insulin in animals fed a high-fat diet (Newgard *et al.* 2009). This observation reflects a difference in metabolic signature for these amino acids between obese and normal weight subjects (Newgard *et al.* 2009). Previously, branched side-chain amino acids (or leucine) were credited instead with an overall benefit on insulin sensitivity, and tolerance with respect to these amino acids is considered good in humans (Baker 2005).

❖ **Hazards often identified**

The list of adverse effects or toxicological hazards that have been demonstrated in animals is long, even though the studies have sometimes not been systematic and with a narrow spectrum. Various journal articles, monographs and expert appraisals have inventoried these different effects (FNB/IOM 2005; AFSSA 2008a; Garlick 2001).
As the identification of these hazards was not followed by a toxicological characterisation study, they remain disparate and ad hoc, and it is difficult to use them to establish an NOAEL (or LOAEL). Moreover, when it does seem possible to establish an NOAEL, the issue of the uncertainty factor has often hampered the calculation of an upper limit. The absence of an upper limit should be considered as due to the lack of data for assessing risk, and not – of course – as an absence of risk, even at moderate doses.

❖ **Conclusion**

The complexity of amino acid metabolism, the scarcity of toxicological data, and the many reasons for the insufficiently characterised risks, lead to the conclusion that it is impossible to conduct a proper risk assessment. This demonstrates the urgent need for a complete set of detailed and high-quality metabolic, physiological and toxicological data, with which to determine the benefits and risks associated with the addition of amino acids to foods for the general population.

4.2.2. Case of carbohydrates (including fibre)

Carbohydrates are a highly heterogeneous family with regard to the size of the molecules involved and their physiological and techno-functional properties. Therefore, only those compounds that have been the subject of a formal request to AFSSA will be used as an example. The fibres, carbohydrates and carbohydrate derivatives previously addressed by the Opinion of 8 September 2008 (AFSSA 2008b), chitosan, chondroitin, fructo-oligosaccharides (FOSs), GABA, glucosamine, inositol, inulin, lactase and pectin, will not be discussed again in this report.
The carbohydrate compounds used as a basis for discussion are as follows: trehalose, galactooligosaccharides (GOS), gamma-cyclodextrin, trans-galactooligosaccharides (TOS), resistant starch (RS), isomaltulose, arabinogalactan, resistant dextrin, D-tagatose, polydextrose, maltodextrin, glucuronolactone and glucosamine hydrochloride.
These compounds are all considered as dietary fibres, or are similar to this category, or are (at least partly) digestible and/or absorbable in the small intestine.
Like all the other ingredients of a compound food, the types of carbohydrates that will be discussed in this Opinion are added to a food:

- for their techno-functional properties:
  - bulking agent (neutral tasting substance which fills volume);
  - gelling thickener;
- for their biological properties:
  - substance having an effect on colon function:
    - prebiotic or presumed as such;
    - likely to activate the digestive tract, either by activating colonic motility, or by an osmotic effect;
    - likely to increase faecal excretion by increasing stool volume, either due to its high capacity for water retention and (relatively) low fermentability or due to its ability to allow the proliferation of colonic microflora;
  - substance with hypoglycaemic (effect in postprandial phase) and/or anticholesteremic potential (chronic effect);
  - scavengers of molecules of interest such as aromas.

A number of the substances considered here are likely to combine several of these properties.

Thus, like all the other substances, carbohydrates have techno-functional, nutritional and/or physiological effects at widely varying doses but these effects also depend on the form in which they occur in foods.

❖ **Definition and classification of carbohydrates (AFSSA 2004c)**

Definition of carbohydrates
The term "carbohydrate" is synonymous with “glucid” and with "saccharide". It relates to polyalcohols with an aldehyde (CHO) or ketone (CO) function. Most monosaccharides and carbohydrate polymers respond respectively to the empirical formulae $(CH_2O)_n$ (where n≥3) and $(C_6H_{10}0_5)_n + (C_5H_8O_4)_m$

Classification of carbohydrates
Carbohydrates are generally classified according to their degree of polymerisation (DP) (**Table 2**) but this classification alone gives no indication of their digestive and metabolic fate (AFSSA 2004c). Thus while some sugars, oligosaccharides and polysaccharides are completely hydrolysed and absorbed in the small intestine, then metabolised in the liver and/or peripheral tissue, carbohydrates of all degrees of polymerisation reach the colon where they are generally fermented or can be excreted in the stool. Finally, many carbohydrates can be only partially digested and/or absorbed in the upper digestive tract and appear partly in the lumen of the colon.

Table 2: Proposed structural classification of the major carbohydrates

Class (DP)	Sub-group	Main components
Sugars (1-2)	Monosaccharides	Glucose, galactose, fructose, tagatose
	Disaccharides	Sucrose, lactose, trehalose, maltose, isomaltulose
Oligosaccharides (3-9)	Malto-oligosaccharides	Maltodextrins
	Other oligosaccharides	Raffinose, stachyose, verbascose, ajugose (α-galactosides), fructo-oligosaccharides, galactooligosaccharides
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches
	Non-starch polysaccharides	Cellulose, hemicelluloses (e.g. galactans, arabinoxylans), pectins, inulin, hydrocolloids (e.g. guar)
Hydrogenated carbohydrates (polyols)	monosaccharide type	Sorbitol, mannitol, xylitol, erythritol
	disaccharide type	Isomalt, lactitol, maltitol
	oligosaccharide type	Maltitol syrups, hydrogenated starch hydrolysates
	polysaccharide type	Polydextrose

Source: Gray, 2003
** This table is itself adapted from the FAO/WHO report (1998). The main change made compared to the FAO/WHO classification is the separation of polyols in a distinct sub-group whereas in the report they appeared as a sub-group of sugars (which therefore only included polyols of DP 1 and 2).*

- **Definition of dietary fibres**

The general formula for fibre is: $(C_6H_{10}O_5)_n + (C_5H_80_4)_m$

AFSSA definition (AFSSA 2002)
Dietary fibre can be:

- either carbohydrate polymers (DP≥3) of plant origin, whether or not associated in the plant with lignin or other non-carbohydrate constituents (polyphenols, waxes, saponins, cutin, phytates, phytosterols, etc.),

- or carbohydrate polymers that are processed (physically, enzymatically or chemically) or synthetic (DP≥3) (see list in AFSSA report published in 2002).

In addition, dietary fibre is neither digested nor absorbed in the small intestine. It has at least one of the following properties:

- Increases production of stools;
- Stimulates colonic fermentation;
- Decreases fasting cholesterol levels;
- Decreases postprandial glycaemia and/or insulin levels.

Codex Alimentarius definition (2008)
Annex II below is added to Directive 90/496/EEC:
"Definition of the material constituting fibre and methods of analysis as referred to in Article 1(4)(j)
For the purposes of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

— edible carbohydrate polymers naturally occurring in the food as consumed;
— edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
— edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence."

As most of the carbohydrate compounds discussed in this Opinion are dietary fibres, the risks associated with their consumption will be discussed in general and the specific features of each fibre of interest will then be mentioned.

❖ **Current and recommended consumption of various dietary fibres**

The first results from INCA2 (2006-2007) compared with those of INCA1 (1998-1999) indicate a stable consumption of fibre in men (18.8 g/d) and a small increase among women (16.4 g/d or a 6.7% increase) (AFSSA 2009a). These levels remain below the recommendations of 25 to 30 g/d. In 2001 the PNNS already recommended "increasing fibre intake by 50% and increasing carbohydrate consumption so that it contributes more than 50% of energy intake".

❖ **Risks associated with the consumption of various dietary fibres**

The physiological effects of dietary fibre are very varied, since, as indicated by the definition (see above), they cover both digestive physiology and therefore intestinal disorders, but also lipid metabolism (Lairon *et al.* 2005). Other possible effects are not explicitly mentioned in the definition; they include for example, prebiotic effects, known for certain oligosaccharides, effects on mineral absorption or the risk of colorectal cancer (Champ *et al.* 2003).
The risks associated with fibre consumption are primarily related to the function of the colon. In particular, they include the risk of digestive discomfort (flatulence, notably) as well as diarrhoea and/or irritation of the digestive tract. The flatulence is related to the rapid production of gas in the colon by bacteria fermenting a substrate that is indigestible by the endogenous enzymes of the digestive tract but highly fermentable; it therefore concerns most monosaccharides, disaccharides or oligosaccharides which are non-digestible and/or absorbable in the small intestine. Diarrhoea can be either osmotic (especially with monosaccharides and disaccharides ingested in large quantities, at once or several times in very close succession) or related to the very high water retention capacity of the fibre (ispaghula, for example). Diarrhoea may also accompany the manifestations of flatulence.
The other risks associated with the consumption of some of these poorly or non-digestible and/or absorbable carbohydrates are:

- risk of mineral deficiencies exacerbated by the consumption of fibre likely to chelate minerals; such risks may be associated with molecules with ionisable functions (carboxylic, for example, as in the case of alginates);
- finally, many of these molecules are obtained through bacteria or purified enzymes. Authorised products currently use bacteria or enzymes permitted in foods, but the possible introduction of new strains and enzymes should be monitored until they have been shown to be safe.

❖ **Risks associated with the consumption of other carbohydrates**

The risks associated with the consumption of certain carbohydrates that are digestible and/or absorbable (at least partially) in the small intestine are as follows **(Table 3**):

- toxicological risk. This is the case with glucuronolactone whose suspected renal toxicity is mentioned in one of the AFSSA reports (energy drink containing this compound) (AFSSA 2003a);
- metabolic risk. Glucosamine (as hydrochloride) has been the subject of more than a dozen studies; two of them concluded that glucosamine has a deleterious effect on glucose metabolism and insulin resistance (Buse 2006);
- these compounds are often synthesised (enzymatically) from sugars extracted from various matrices. When the original product carries allergic risks, precautionary labelling indicating the possible presence of these proteins should be required. This is the case, for example, with D-tagatose obtained from lactose of milk origin;
- as with fibres, many of these compounds are obtained through bacteria or purified enzymes whose safety must be tested if it has not yet been established;
- Finally, some of these compounds are glycaemic (e.g. isomaltulose, γ-cyclodextrin). Information is necessary for patients with diabetes.

As with many ingredients, there are no consumption data for most of the compounds discussed in this report. In addition, few or no quantitative data exist on the risks, even minor, associated with these compounds. When they are available, they are given in the **Table in Annex II.**

4.2.3. Case of CLAs

❖ **Definition, origin and intakes**

The conjugated isomers of linoleic acid (conjugated linoleic acids, or CLAs) are *trans* fatty acids formed during ruminal biohydrogenation, which, after absorption, are found in tissues and milk. They are known as "natural" *trans* fatty acids.
The main food contributors to CLA intake are dairy products, with levels ranging from 0.3 to 0.8%. In these foods, the major CLA isomer is 9c,11t or rumenic acid which accounts for 87% of CLAs. The other isomer, 10t,12c, accounts for 4.5% of CLAs (AFSSA 2005a).
CLAs may also come from the processing of fats used in the manufacture of many products such as croissant-like pastries and biscuits. Levels range from 0.3 to 0.5% of total fatty acids. In this case, the predominant forms of CLA are 9t,11t and 10t,12t, while rumenic acid, 9c,11t, accounts for only 5-8% of CLAs.
Finally, CLAs can be synthesised for incorporation into food supplements. Syntheses lead to mixtures of 9c,11t and 10t,12c isomers which are found in similar amounts.
The highest contributors to CLA intake in adults are in descending order: butter, cheese, croissant-like pastries, meat, milk and fresh dairy products. According to the AFSSA report, CLA intake is around 200 mg/d in adults, with consumption ranging from 60 to over 400 mg/d. It can therefore be estimated that intakes of 9c,11t would be no more than 180 mg/d and 9 mg/d for 10t,12c (AFSSA 2005a).
In terms of public health, consumption of total *trans* fatty acids significantly increases cardiovascular risks and a consumption threshold not to be exceeded of 2% of total energy intake (TEI) has been proposed. This value includes *trans* fatty acids of animal and technological origin and remains above the average estimated consumption (less than 1% TEI) (AFSSA, 2009c). Concerning CLAs, no threshold has been proposed in view of their low consumption (6% of total *trans* acids) (AFSSA 2005a).
In humans, very few studies have examined the effect of the separated isomers, at least until 2004. Studies focus on a synthetic mixture of 9c,11t and 10t,12c in equivalent quantities and at high doses, as there is no natural analogue of this mixture with a similar ratio.

❖ **CLA supplementation**

It has been shown that CLAs alter body composition and lead to a reduction in body fat and an increase in lean body mass in various animal species (Evans *et al.* 2002). Because of a potentially beneficial effect on the regulation of body fat, intervention studies have been conducted using food supplements. It can be considered that in most studies, CLA supplementation is up to 3.4 g/d in humans with doses ranging from 1.4 g/d (Mougios *et al.* 2001) to 6.8 g/d (Blankson *et al.* 2000). The CLAs incorporated into food supplements correspond to a mixture of two isomers, 9c,11t and 10t,12c, in equivalent quantities, providing 1.7 g of each isomer. The AFSSA report concluded that the effect of CLAs in reducing body fat is not proven in humans, casting doubt on the beneficial effect of the mixture of CLAs. In animals, while supplementation does lead to lipoatrophy, many deleterious effects have been demonstrated (AFSSA 2005a).

Toxic effects in animals

The toxic effects depend on the species, but studies in mice, hamsters, rats, and pigs generally show that CLAs reduce fat deposition (AFSSA 2005a). This effect is not found in obese rats, in which CLA supplementation has no effect on body fat and even increased it after 5 weeks of experimentation (Sisk *et al.* 2001). The 10t,12c isomer is considered responsible for the reduction in lipid storage. However, this effect is tissue specific. Indeed, this isomer increases lipid content in many other tissues (muscle and liver), causes insulin resistance and increases insulin levels. This effect is particularly pronounced in mice since it leads to hepatomegaly, hepatic steatosis and lipodystrophic diabetes, with supplementation of 0.4% w/w of food for 4 weeks (Clement *et al.* 2002). Fortifying the diet with the 9c,11t isomer or with linoleic acid has no significant effect on lipid storage. However a recent study showed that insulin resistance is induced not only by administration of the 10t,12c isomer for 6 months but also by the mixture 9c, 11t/10t, 12c, whereas the

9c,11t isomer prevents it (Halade *et al.* 2010). This suggests that the presence of the 9c,11t isomer does not offset the deleterious effects of 10t,12c.

- Absence of genotoxic effect *in vitro:* EFSA concluded, in recent opinions, that the available data (provided by the applicants for the evaluation of specific products) do not report any genotoxic effects of CLAs (racemic mixtures of 9c,11t/10t,12c isomers) (EFSA, 2010b, EFSA, 2010a).

In conclusion, an intake of about 0.5% of CLAs in the diet causes hepatotoxic and diabetogenic effects. From a 90-day toxicological study, an NOAEL of 2.43 g/kg/d in males and 2.73 g/kg/d in females was determined in rats (O'Hagan and Menzel 2003).

Deleterious effects in humans
The doses used for supplementation in humans show that with a daily dose of about 1.7 g of each isomer provided by a capsule, consumption is increased by a factor of ten for 9c,11t and of 200 for 10t,12c. While the beneficial effect on body composition has not been proven, various adverse effects have been reported:

- atherogenic effect: this effect was found in several studies showing a decrease in HDL-C (4 to 14%) after 8 to 12 weeks of supplementation with a mixture of 9c,11t and 10t,12c (Tholstrup *et al.* 2008) or supplementation with 10t,12c (Riserus *et al.* 2002a; Tricon *et al.* 2004);
- diabetogenic effect: the insulin sensitivity measured using a euglycemic clamp was reduced by 19% after 12 weeks of supplementation with the 10t,12c isomer (Riserus *et al.* 2002a);
- effect on markers of inflammation: induction of lipid peroxidation was more pronounced after 12 weeks of supplementation with the 10t,12c isomer than after 12 weeks of supplementation with the mixture (Riserus *et al.* 2002b); increased C-reactive protein (CRP) and plasminogen activator inhibitor 1 (PAI 1) levels were observed after 16 weeks of supplementation with the mixture of the two isomers but not with the 9c,11t isomer (Tholstrup *et al.* 2008);
- effect on oxidative stress: a mixture of 9c, 11t and 10t,12c isomers *in vitro* associated with an increase in ROS synthesis and isoprotane production by macrophages (Stachowska *et al.* 2008);
- effect on endothelial function: decreased production of nitric oxide *in vitro* by each of the two isomers (Eder *et al.* 2003).

❖ **Conclusion**

Ultimately, the effect of a mixture of CLAs on reducing body fat remains to be proven in humans. The recent EFSA opinions on health claims relating primarily to the maintenance of body weight, the increase in lean mass and increased insulin sensitivity confirm this (EFSA 2010). Many adverse effects have been observed in animals and humans, especially in diabetic patients. In recent opinions, EFSA concluded as to the absence of risk associated with consumption (for 6 months) of two products containing a racemic mixture of 9c,11t and 10t,12c, indicating that this safety does not apply to subjects with type 2 diabetes. ANSES reiterates its concerns about the fortification of foods with CLAs, given the lack of demonstrated long-term safety for the general population and the proven deleterious effect of the 10t,12c isomer (AFSSA 2005a).

4.2.4. Case of phytoestrogens

Phytoestrogens are substances occurring naturally in plants or derived from the metabolism of an ingested plant precursor by colonic flora. These molecules have a similar chemical structure to 17β-estradiol which is the reason for their functional similarities. As a result, the intake of these substances has been proposed in certain physiological situations or in the prevention of certain conditions. This aspect will be reviewed briefly before describing in greater detail the possible deleterious effects associated with the addition of these substances to common food products.

❖ **Definition, metabolism and physiological effects**

- *Definition*

For a compound to be listed as a phytoestrogen, its activity must have been demonstrated *in vivo* by testing devoted to the demonstration of estrogenic effects (OECD uterotrophic and vaginal cornification tests). In the case of *in vitro* tests, compounds are considered as estrogenic if the effects are observed at doses equivalent to the plasma concentrations observed after normal dietary intake.

Phytoestrogens can be divided into fourteen compounds belonging to six chemical groups (isoflavones, isoflavanes, coumestans, flavanones, chalcones, lignans and enterolignans). Certain plant extracts that are sources of phytoestrogens have also exhibited estrogenic activity (clover, fennel, flax and liquorice). The soy isoflavones, and among them genistein and daidzein, have been most studied because they contribute to almost all phytoestrogen intake in Asian women. In the West, apart from people consuming soy-based products (about 1% of the population in France) (AFSSA 2005b), phytoestrogen intake comes mainly from foods containing enterolignan precursors. Many plant products contain them but berries, sesame seeds and linseed are the most important sources and to a lesser extent, vegetables rich in carotenoids and cruciferous vegetables. However, increasingly, flour made from soy and other legumes (beans) is used as an ingredient in various foods, which may lead to a rise in phytoestrogen consumption. Due to the lack of complete consumption and composition data however, this consumption is difficult to estimate.

- *Metabolism*

By the oral route, phytoestrogens reach the intestinal lumen mainly in the form of glycosides, then the bloodstream in the form of aglycones. This transformation involves enterocyte enzymes but also the colonic flora, which can promote the metabolism of the glycoside form into aglycone and then into other metabolites, which may be more active: this is the case of daidzein (glycoside), which is metabolised into daidzein (aglycone) and then equol. This metabolism varies from one species to another (e.g. it is greater in rats than in humans) and from one population group to another, and may vary depending on diet (mainly fibre intake). The metabolites of phytoestrogens then bind to the β receptors and to a lesser extent the α receptors of estrogens, but with a lower affinity than that of specific ligands, particularly β-estradiol. The ingestion of these compounds usually has a modest, lowering effect on hormone levels. A lengthening of the menstrual cycle, from the increase in the follicular phase, is frequently found. Finally, some studies have observed increased metabolism of estrogens into non-genotoxic catechol estrogens (2-OH), especially after supplementation in lignans. This would confirm the hormonal effect of phytoestrogens, which varies according to the tissues and doses considered.

- *Physiological effects*

Several studies have explored the effect of phytoestrogen intake on menopausal symptoms (hot flushes, vaginal dryness). A recent collective expert appraisal (Loprinzi *et al.* 2008) concluded, after reviewing randomised controlled trials, as to the lack of a substantial effect of phytoestrogens on hot flushes, confirming AFSSA's previous findings (AFSSA 2005b).

As for osteoporosis, while the epidemiological data in 2005 were insufficient to reach a conclusion, the large randomised, placebo-controlled multicenter PHYTOS trial (Brink *et al.* 2008), involving 237 healthy postmenopausal women, showed that daily consumption of 110 mg of isoflavones for one year had no effect on bone loss, formation or resorption.

Regarding the effects of soy isoflavones on cognitive function and aging, the review by Zhao (Zhao and Brinton 2007) shows that of eight intervention studies, four had a beneficial effect. Thus, despite new results, it is not possible to go beyond the findings issued in 2005, that more rigorous studies are needed to reach a conclusion.

With regard to cancer, the most numerous studies concern breast cancer among Asian women for whom a decreased risk was regularly found, although the dietary context of these women

corresponds to a diet typically associated with reduced risk, suggesting an effect related to confounding factors. Just one study, which introduced various dietary factors in the multivariate analysis, did not reveal any reduction in the risk associated with consumption of soy-based products (Nishio *et al.* 2007). Work is needed to assess the effects of phytoestrogens in Caucasian women. It is therefore not currently possible to attribute a convincing or probable level of evidence[4] to phytoestrogens in preventing breast cancer. There are fewer studies for prostate cancer, but the results are more consistent, including in multi-ethnic cohorts. After adjustment for various dietary factors, these studies are consistent with a reduced risk, particularly in Caucasian populations, for high intakes of enterolignan precursors, and the level of evidence can be described as possible (Yan and Spitznagel 2009). For other cancers, there are not enough studies to be able to reach any conclusion.
With regard to the cardiovascular system, the data are not sufficient to conclude on a specific cholesterol-lowering effect of phytoestrogens (AFSSA 2005b).

❖ **Toxicity, reproductive system and carcinogenicity**

The AFSSA report (2005) concluded: "Studies of toxicity by repeated administration, of genotoxicity, carcinogenicity, and also studies of fertility, sexual organ development and maturation, have been conducted mostly in rodents, rarely in dogs and monkeys. Phytoestrogens appear devoid of general toxicity but may be genotoxic or carcinogenic in some animal models and *in vitro*. The value above which the potential toxicity of isoflavones has not been sufficiently documented for use in humans is 1 mg/kg bw/d, and this figure has been provisionally adopted as the safety limit. Furthermore, regular *in utero* or perinatal exposure is accompanied by alterations in sexual organ development, maturity and sometimes also fertility."

- *Toxicity studies in rodents*

In 2008, the "National Toxicology Program" published its conclusions from classic toxicology (National Toxicology Program 2008a) and carcinogenicity studies (National Toxicology Program 2008b) in Sprague-Dawley rats:

Effect on reproduction:
Genistein induces abnormalities in the development of reproductive organs for exposures to 100 or 500 ppm, doses which correspond to intakes of approximately 7 and 35 mg/kg of body weight per day for males and 10 and 51 mg/kg of body weight per day for females (vaginal opening accelerated, accentuated in females, gynaecomastia and delayed testicular descent in males). However, no deleterious effects on the reproductive system were observed for genistein concentrations ranging from 1 to 100 ppm, which correspond to the expected serum levels from human food intake. Some clearly toxic effects were transmitted through the directly exposed generations and appeared in offspring which were not exposed under the exposure conditions of the study (100 and 500 ppm).

Carcinogenicity:
In males, no carcinogenic effect was observed for exposures to 5, 100 or 500 ppm. In females subjected to the same dose, there was a marginal increase in pituitary gland tumours, adenomas and mammary adenocarcinomas. Changes in oestrus were also observed. These effects in females appear to be directly related to oestrogen toxicity.
In the event that phytoestrogens do have a carcinogenic effect, this would occur rather at the tumour promotion and growth phases, and therefore tests exploring precisely these stages of carcinogenesis are needed to assess their carcinogenicity.

- *Experimental studies and observations in humans*

These studies investigated the effect of exposure to phytoestrogens in infants or young children through the consumption of tonyu (soy milk). On this point, AFSSA concluded in 2005: "Four-month old infants fed exclusively from birth with preparations based on soy protein may receive from 4 to 9 mg/kg bw/d of isoflavones. In view of the toxicity studies, we must emphasise the potential risk on growth, endocrine development, onset of puberty and fertility, as well as immunity and thyroid function, related to the consumption of such preparations by infants and young children, even

[4] With reference to the three levels of evidence defined by the WCRF, "convincing", "probable" and "limited but suggestive" (2007)

though there are no corresponding human studies. Children consuming tonyu and/or soy-based desserts can also receive high amounts of isoflavones exceeding 1 mg/kg bw/day". AFSSA therefore recommended avoiding the consumption of such preparations by children under 3 years, at least while their isoflavone content is unknown.
Since then, three studies have provided arguments for this restriction: an experimental study in marmosets (Tan *et al.* 2006) and two observational studies in humans (Chavarro *et al.* 2008; Milerova *et al.* 2006):

- the study by Tan *et al.* was conducted in seven sets of 4-5 day old marmoset twins of which some received soy formulae, and others the standard formula for 5 to 6 weeks (Tan *et al.* 2006). Blood samples were taken every 10 weeks for hormonal tests and animals were sacrificed at 120-138 weeks to perform histological examinations. No adverse effects were observed on the reproductive system, other than an alteration of the size and cellular composition of the testes, indicating indirectly but consistently a possible compensation for impairment of Leydig cells;
- the study by Chavarro *et al.* was conducted in 99 men recruited from sub-fertile couples who were asked about consumption of 15 soy-containing foods over the previous three months (Chavarro *et al.* 2008). The association of this consumption with the sperm's qualitative and quantitative characteristics was explored by linear regression and by quantile. The results show that there is an inverse relationship between consumption of soy-containing foods and sperm count, demonstrated after adjustment for individual confounding factors (-41 million/mL for consumption ≥ 2 servings/week compared with a lack of consumption);
- the study by Milerova *et al.* sought, in 268 children screened for an iodine deficiency, but without clinical signs, a correlation between circulating levels of isoflavones and thyroid hormone function (Milerova *et al.* 2006). A significant positive association of genistein with thyroglobulin autoantibodies and a negative correlation with thyroid volume were observed. A multiple regression of the relationship between isoflavone levels and thyroid parameters showed a small but significant association between genistein and thyroid variables. Such an effect should therefore be considered in cases of iodine deficiency.

Thus, the results of all these studies, which need to be reinforced, support the recommendations for precautions issued by AFSSA in 2005 (AFSSA 2005b).

❖ **Conclusion**

AFSSA's recommendations (2005) on safety related to consumption of phytoestrogens appear reinforced by recent studies, in particular with regard to growth, endocrine development, onset of puberty and fertility, and thyroid function. However, other observational studies, especially taking into account age and duration of exposure, are still needed.
Some populations have a level of intake that makes such studies possible. However, the difficulty remains the measurement of exposure to phytoestrogens, due to the scarcity of composition tables and the lack of labelling of foods containing soy or its derivatives. In addition, soy-based foods consumed in Western countries cannot be considered similar to soy-based foods eaten in Asian countries, as the preparation methods and other ingredients in the food are different. Analysis of composition and nutritional surveys are therefore needed to clarify the exposure of subjects. The results of this work may enable the threshold value of isoflavones to be defined, from which labelling of their level in foods would be required, as recommended in the AFSSA report on nutrition labelling (AFSSA 2008e), which also proposed that restrictions for at-risk populations be mentioned.
Animal studies therefore remain fully relevant to assessing the toxicity of phytoestrogens, particularly to the development of sexual characteristics and fertility, but it is necessary to monitor their relevance in terms of carcinogenicity and to consider the various mechanisms, proven or suspected, that may affect carcinogenesis: effect on metabolism and bioavailability of sex hormones, tumour promotion and proliferation.

GENERAL CONCLUSION

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) selected four classes of substances to illustrate the complexity of the assessment of risks associated with fortification in "other substances": amino acids, fibre or other carbohydrates, conjugated linoleic acids (CLAs) and phytoestrogens. These examples were chosen because of the existence of risk factors discussed in the literature and any other warning data. In addition, they reflect the market reality of food fortification and food supplements. Several critical points emerge from this assessment:

- The complexity of amino acid metabolism and the overlapping in many signalling pathways, the lack of toxicological data despite many hazards being identified;
- The difficulty of extrapolating from one population group to another, especially due to a different metabolism of substances (effects of different phytoestrogens in the Asian and European populations) or genetic polymorphism within the same population (amino acids);
- The need to conduct a risk assessment specific to each of the substances belonging to the same group (different CLA isomers, different types of fibres).

In general, ANSES considers that fortifying commonly consumed foods with substances with nutritional or physiological effects ("other substances") may only be considered in the following two cases:

- if there is inadequate intake in the general population or in a sub-group of the population of nutrients for which there are population reference intakes (ANCs);
- if a specific nutritional/physiological benefit is demonstrated.

In these situations, an assessment is needed of the risks associated with the addition of vitamins and minerals and "other substances" to foods. They can be of various types: intrinsic risks, risks associated with changes in bioavailability and with effects of food matrices, risks associated with secondary metabolites, risks associated with production processes, risks associated with interactions between substances and between drugs and substances, risks of over-exposure due to the increase in food sources, risks associated with specific characteristics of certain sub-populations, behavioural risks, etc.

As a reminder, the approach taken by AFSSA for fortification in vitamins and minerals was based on maximum consumption data and the safety limit, in order to facilitate the determination of a maximum fortification limit guaranteeing an acceptable level of safety. But in the case of these "other substances", in most cases no nutritional reference value (ANC or safety limit) has been defined and/or no consumption data are available. ANSES considers that these consumption data should include all food sources for the "other substances", including intakes from food supplements. Thus, the absence of a safety limit, or failing this a maximum intake limit, should be considered as being due to the lack of data for assessing risk, and not as an absence of risk, even at moderate doses.

ANSES therefore reiterates that a case-by-case assessment of pairs of “other substances” and food vectors, under precise fortification conditions (specifically the dose) is needed to ensure maximum safety to the consumer in terms of the available data. ANSES also believes that this risk assessment in view of possible fortification should be combined with a benefit assessment.

The Director General

Marc MORTUREUX

KEY WORDS

Key words: Regulation 1925/2006, fortification, benefits, risks, amino acids, CLAs, phytoestrogens, fibre, carbohydrates

COMPOSITION OF THE DELIBERATION GROUP

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REFERENCES

AFSSA (2002) Les fibres alimentaires: définition, méthodes de dosage, allégations nutritionelles.

AFSSA (2003a) Avis du 5 mai 2003 relatif à l'évaluation de à l'emploi de taurine, D-glucuronolactone, de diverses vitamines et de caféine (à une dose supérieure à celle actuellement admise dans les boissons) dans une boisson dite "énergétique".

AFSSA (2003b) Evaluation de la sécurité, de l'intérêt et de l'allégation des denrées alimentaires, contenant des plantes destinées à la consommation humaine.

AFSSA (2004a) avis du 18 octobre 2004 relatif aux compléments d'information fournis après la publication de l'avis relatif à l'emploi de lycopène dérivé de Blakeslea trispora,comme ingrédient alimentaire.

AFSSA (2004b) Cahier des charges pour le couple nutriment-aliment vecteur.

AFSSA (2004c) Glucides et Santé - Etat des lieux, évaluation et recommandations.

AFSSA (2005a) Risques et bénéfices pour la santé des acides gras *trans* apportés par les aliments.

AFSSA (2005b) Sécurité et bénéfices des phyto-estrogènes apportés par l'alimentation - Recommandations.

AFSSA (2007a) Avis du 7 mai 2007relatif aux types de constituants glucidiques à introduire dans le dispositif de surveillance des compositions et des apports glucidiques.

AFSSA (2007b) Avis du 10 janvier 2007 relatif aux lignes directrices pour la constitution et l'évaluation de dossiers portant sur les allégations nutritionnelles et de santé revendiquées pour les denrées alimentaires.

AFSSA (2007c) Avis du 14 décembre 2007 relatif à l'emploi dans les compléments alimentaires de plantes ayant fait l'objet de signalements de pharmacovigilance.

AFSSA (2007d) Avis du 21 décembre 2007 relatif à l'évaluation d'un projet d'arrêté relatif à l'emploi de substances à but nutritionnel ou physiologique et de plantes et préparations de plantes dans la fabrication des compléments alimentaires.

AFSSA (2008a) Apport en protéines: consommation, qualité, besoins et recommandations. AFSSA.

AFSSA (2008b) Avis du 8 septembre 2008 relatif à l'évaluation d'un projet d'arrêté relatif à l'emploi de substances à but nutritionnel ou physiologique et de plantes et préparations de plantes dans la fabrication de compléments alimentaires.

AFSSA (2008c) Avis du 16 mai 2008 relatif à l'évaluation d'un projet relatif à l'emploi de substances à but nutritionnel ou physiologique et de plantes et préparations de plantes dans les compléments alimentaires.

AFSSA (2008d) Avis du 20 juin 2008 relatif à l'évaluation d'un projet d'arrêté relatif à l'emploi de substances à but nutritionnel ou physiologique et de plantes et préparations de plantes dans la fabrication de compléments alimentaires.

AFSSA (2008e) Modification de l'étiquetage nutritionnel: proposition, arguments et pistes de recherche.

AFSSA (2009a) Etude Individuelle Nationale des Consommations Alimentaires 2 (INCA 2).

AFSSA (2009b) Nanotechnologies et nanoparticules dans l'alimentation humaine et animale.

Alles, M S, Hartemink, R, Meyboom, S, Harryvan, J L, Van Laere, K M, Nagengast, F M & Hautvast, J G (1999) Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. Am J Clin Nutr, 69**,** 980-91.

Atbc (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med, 330**,** 1029-35.

Baker, D H (2005) Tolerance for branched-chain amino acids in experimental animals and humans. J Nutr, 135**,** 1585S-90S.

Bier, D M (2003) Amino acid pharmacokinetics and safety assessment. J Nutr, 133**,** 2034S-2039S.

Bjelakovic, G, Nikolova, D, Gluud, L L, Simonetti, R G & Gluud, C (2008) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev**,** CD007176.

Blankson, H, Stakkestad, J A, Fagertun, H, Thom, E, Wadstein, J & Gudmundsen, O (2000) Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J Nutr, 130**,** 2943-8.

Brink, E, Coxam, V, Robins, S, Wahala, K, Cassidy, A & Branca, F (2008) Long-term consumption of isoflavone-enriched foods does not affect bone mineral density, bone metabolism, or

hormonal status in early postmenopausal women: a randomized, double-blind, placebo controlled study. Am J Clin Nutr, 87, 761-70.
Buse, M G (2006) Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab, 290, E1-E8.
Champ, M, Langkilde, a M, Brouns, F, Kettlitz, B & Collet Yle, B (2003) Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. Nutr Res Rev, 16, 71-82.
Chavarro, J E, Toth, T L, Sadio, S M & Hauser, R (2008) Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic. Hum Reprod, 23, 2584-90.
Clement, L, Poirier, H, Niot, I, Bocher, V, Guerre-Millo, M, Krief, S, Staels, B & Besnard, P (2002) Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res, 43, 1400-9.
Eder, K, Schleser, S, Becker, K & Korting, R (2003) Conjugated linoleic acids lower the release of eicosanoids and nitric oxide from human aortic endothelial cells. J Nutr, 133, 4083-9.
EFSA (2009a) EFSA Scientific Cooperation (ESCO) Working Group on Botanicals and Botanical Preparations; Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies on request of EFSA. EFSA Journal, 7, 280-384. www.efsa.europa.eu.
EFSA (2009b) ESCO Report on Analysis of Risks and Benefits of Fortification of Food with Folic Acid
EFSA (2009c) The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. Scientific Opinion of the Scientific Committee
(Question No EFSA-Q-2007-124a) Adopted on 10 February 2009.
EFSA (2009d) The use of taurine and D-gluuronolactone as constituents of the so-called "energy drinks" - Scientific opinion of the panel on food additives and nutrient sources added to food. EFSA Journal, 935, 1-31.
EFSA (2010) Scientific Opinion on the substantiation of health claims related to conjugated linoleic acid (CLA) isomers and contribution to the maintenance or achievement of a normal body weight (ID 686, 726, 1516, 1518, 2892, 3165), increase in lean body mass (ID 498, 731), increase in insulin sensitivity (ID 1517), protection of DNA, proteins and lipids from oxidative damage (ID 564, 1937), and contribution to immune defences by stimulation of production of protective antibodies in response to vaccination (ID 687, 1519) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal, 8, 1794.
Elliott, R, Pico, C, Dommels, Y, Wybranska, I, Hesketh, J & Keijer, J (2007) Nutrigenomic approaches for benefit-risk analysis of foods and food components: defining markers of health. Br J Nutr, 98, 1095-100.
Evans, M, Brown, J & Mcintosh, M (2002) Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. J Nutr Biochem, 13, 508.
Fao/Who (2006) A model for establishing upper levels of intake for nutrients and related substances. Geneva, FAO/WHO Technical Workshop on Nutrient Risk Assessment.
Flood, M T, Auerbach, M H & Craig, S A (2004) A review of the clinical toleration studies of polydextrose in food. Food Chem Toxicol, 42, 1531-42.
Fnb/Iom (2005) Protein and Amino acids. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, D.C., The National Academies Press.
Garlick, P J (2001) Assessment of the safety of glutamine and other amino acids. J Nutr, 131, 2556S-61S.
Grimble, R F (2006) The effects of sulfur amino acid intake on immune function in humans. J Nutr, 136, 1660S-1665S.
Gueguen, L & Pointillart, A (2000) The bioavailability of dietary calcium. J Am Coll Nutr, 19, 119S-136S.
Halade, G V, Rahman, M M & Fernandes, G (2010) Differential effects of conjugated linoleic acid isomers in insulin-resistant female C57Bl/6J mice. J Nutr Biochem, 21, 332-7.
Hasler, C M (2008) Health claims in the United States: an aid to the public or a source of confusion? J Nutr, 138, 1216S-20S.
Hathcock, J N & Shao, A (2008) Expanded approach to tolerable upper intake guidelines for nutrients and bioactive substances. J Nutr, 138, 1992S-1995S.
Hesketh, J, Wybranska, I, Dommels, Y, King, M, Elliott, R, Pico, C & Keijer, J (2006) Nutrient-gene interactions in benefit-risk analysis. Br J Nutr, 95, 1232-6.

Horwitt, M K, Harper, a E & Henderson, L M (1981) Niacin-tryptophan relationships for evaluating niacin equivalents. Am J Clin Nutr, 34**,** 423-7.

Koutsou, G A, Storey, D M & Bar, A (1999) Gastrointestinal tolerance of gamma-cyclodextrin in humans. Food Addit Contam, 16**,** 313-7.

Lairon, D, Arnault, N, Bertrais, S, Planells, R, Clero, E, Hercberg, S & Boutron-Ruault, M C (2005) Dietary fiber intake and risk factors for cardiovascular disease in French adults. Am J Clin Nutr, 82**,** 1185-94.

Legrand, P, Bourre, J, Descomps, B, Durand, G & Renaud, S (2001) Apports Nutritionnels Conseillés pour la population française. Lipides. IN MARTIN, A. (Ed.).

Lina, B A, Jonker, D & Kozianowski, G (2002) Isomaltulose (Palatinose): a review of biological and toxicological studies. Food Chem Toxicol, 40**,** 1375-81.

Loprinzi, C L, Barton, D L, Sloan, J A, Novotny, P J, Dakhil, S R, Verdirame, J D, Knutson, W H, Kelaghan, J & Christensen, B (2008) Mayo Clinic and North Central Cancer Treatment Group hot flash studies: a 20-year experience. Menopause, 15**,** 655-60.

Mariotti, F, Kalonji, E, Huneau, J F & Margaritis, I (2010) Potential pitfalls of health claims from a public health nutrition perspective. Nutr Rev, 68**,** 624-38.

Milerova, J, Cerovska, J, Zamrazil, V, Bilek, R, Lapcik, O & Hampl, R (2006) Actual levels of soy phytoestrogens in children correlate with thyroid laboratory parameters. Clin Chem Lab Med, 44**,** 171-4.

Morris, S M, Jr. (2007) Arginine metabolism: boundaries of our knowledge. J Nutr, 137**,** 1602S-1609S.

Mougios, V, Matsakas, A, Petridou, A, Ring, S, Sagredos, A, Melissopoulou, A, Tsigilis, N & Nikolaidis, M (2001) Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. J Nutr Biochem, 12**,** 585-594.

Munro, I C, Newberne, P M, Young, V R & Bar, A (2004) Safety assessment of gamma-cyclodextrin. Regul Toxicol Pharmacol, 39 Suppl 1**,** S3-13.

National Toxicology Program (2008a) NTP Multigenerational Reproductive Study of Genistein (CAS No. 446-72-0) in Sprague-Dawley Rats (Feed Study). Natl Toxicol Program Tech Rep Ser**,** 1-266.

National Toxicology Program (2008b) NTP Toxicology and Carcinogenesis Studies of Genistein (CAS No. 446-72-0) in Sprague-Dawley Rats (Feed Study). Natl Toxicol Program Tech Rep Ser**,** 1-240.

Newgard, C B, An, J, Bain, J R, Muehlbauer, M J, Stevens, R D, Lien, L F, Haqq, a M, Shah, S H, Arlotto, M, Slentz, C A, Rochon, J, Gallup, D, Ilkayeva, O, Wenner, B R, Yancy, W S, Jr., Eisenson, H, Musante, G, Surwit, R S, Millington, D S, Butler, M D & Svetkey, L P (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab, 9**,** 311-26.

Nishio, K, Niwa, Y, Toyoshima, H, Tamakoshi, K, Kondo, T, Yatsuya, H, Yamamoto, A, Suzuki, S, Tokudome, S, Lin, Y, Wakai, K, Hamajima, N & Tamakoshi, A (2007) Consumption of soy foods and the risk of breast cancer: findings from the Japan Collaborative Cohort (JACC) Study. Cancer Causes Control, 18**,** 801-8.

O'hagan, S & Menzel, A (2003) A subchronic 90-day oral rat toxicity study and in vitro genotoxicity studies with a conjugated linoleic acid product. Food Chem Toxicol, 41**,** 1749-60.

Omenn, G S, Goodman, G E, Thornquist, M D, Balmes, J, Cullen, M R, Glass, A, Keogh, J P, Meyskens Jr, F L, Valanis, B, Williams Jr, J H, Barnhart, S & Hammar, S (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. New Engl J Med, 334**,** 1150-1155.

Oxenkrug, G F (2007) Genetic and hormonal regulation of tryptophan kynurenine metabolism: implications for vascular cognitive impairment, major depressive disorder, and aging. Ann N Y Acad Sci, 1122**,** 35-49.

Pencharz, P B, Elango, R & Ball, R O (2008) An approach to defining the upper safe limits of amino acid intake. J Nutr, 138**,** 1996S-2002S.

Raitala, A, Pertovaara, M, Karjalainen, J, Oja, S S & Hurme, M (2005) Association of interferon-gamma +874(T/A) single nucleotide polymorphism with the rate of tryptophan catabolism in healthy individuals. Scand J Immunol, 61**,** 387-90.

Renwick, a G (2004) Establishing the upper end of the range of adequate and safe intakes for amino acids: a toxicologist's viewpoint. J Nutr, 134**,** 1617S-1624S; discussion 1630S-1632S, 1667S-1672S.

Renwick, a G (2006) Toxicology of micronutrients: adverse effects and uncertainty. J Nutr, 136**,** 493S-501S.

Richards, a B, Krakowka, S, Dexter, L B, Schmid, H, Wolterbeek, a P, Waalkens-Berendsen, D H, Shigoyuki, A & Kurimoto, M (2002) Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. Food Chem Toxicol, 40**,** 871-98.

Riserus, U, Arner, P, Brismar, K & Vessby, B (2002a) Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care, 25**,** 1516-21.

Riserus, U, Basu, S, Jovinge, S, Fredrikson, G N, Arnlov, J & Vessby, B (2002b) Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation, 106**,** 1925-9.

Sakai, R, Miura, M, Amao, M, Kodama, R, Toue, S, Noguchi, Y & Kimura, T (2004) Potential approaches to the assessment of amino acid adequacy in rats: a progress report. J Nutr, 134**,** 1651S-1655S; discussion 1664S-1666S, 1667S-1672S.

Shao, A & Hathcock, J N (2008) Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regul Toxicol Pharmacol, 50**,** 376-99.

Sisk, M B, Hausman, D B, Martin, R J & Azain, M J (2001) Dietary conjugated linoleic acid reduces adiposity in lean but not obese Zucker rats. J Nutr, 131**,** 1668-74.

Stachowska, E, Baskiewicz-Masiuk, M, Dziedziejko, V, Gutowska, I, Baranowska-Bosiacka, I, Marchlewicz, M, Dolegowska, B, Wiszniewska, B, Machalinski, B & Chlubek, D (2008) Conjugated linoleic acid increases intracellular ROS synthesis and oxygenation of arachidonic acid in macrophages. Nutrition, 24**,** 187-99.

Tan, K A, Walker, M, Morris, K, Greig, I, Mason, J I & Sharpe, R M (2006) Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. Hum Reprod, 21**,** 896-904.

Tholstrup, T, Raff, M, Straarup, E M, Lund, P, Basu, S & Bruun, J M (2008) An oil mixture with trans-10, cis-12 conjugated linoleic acid increases markers of inflammation and in vivo lipid peroxidation compared with cis-9, trans-11 conjugated linoleic acid in postmenopausal women. J Nutr, 138**,** 1445-51.

Tricon, S, Burdge, G C, Kew, S, Banerjee, T, Russell, J J, Jones, E L, Grimble, R F, Williams, C M, Yaqoob, P & Calder, P C (2004) Opposing effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on blood lipids in healthy humans. Am J Clin Nutr, 80**,** 614-20.

Van Can, J G, Ijzerman, T H, Van Loon, L J, Brouns, F & Blaak, E E (2009) Reduced glycaemic and insulinaemic responses following isomaltulose ingestion: implications for postprandial substrate use. Br J Nutr, 102**,** 1408-13.

Watford, M (2008) Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. J Nutr, 138**,** 2003S-2007S.

Who (2001) Safety evaluation of certain food. Gamma-cyclodextrin. WHO Food Additive Series, 42**,** 183-202.

Wisker, E (2003) [Probiotics]. Zentralbl Gynakol, 125**,** 475-9.

Yan, L & Spitznagel, E L (2009) Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. Am J Clin Nutr, 89**,** 1155-63.

Zentek, J, Marquart, B & Pietrzak, T (2002) Intestinal effects of mannanoligosaccharides, transgalactooligosaccharides, lactose and lactulose in dogs. J Nutr, 132**,** 1682S-4S.

Zhao, L & Brinton, R D (2007) WHI and WHIMS follow-up and human studies of soy isoflavones on cognition. Expert Rev Neurother, 7**,** 1549-64.

Zijp, I M, Korver, O & Tijburg, L B M (2000) Effect of tea and other dietary factors on iron absorption. Crit Rev Food Sci Nutr, 40**,** 371-98.

ANNEXES

Annex 1. List of substances that have been the subject of AFSSA Opinions

Substance	Formal request	Limit/information on the risk	**Expert appraisal under way**
Galacto-oligosaccharide (GOS)	2000-SA-0332 2000-SA-0147b	Mixture of FOS+GOS 0.8 g/dL of product accepted in infant formula.	**See also this Opinion**
Gamma-cyclodextrin	2001-SA-0119 (additive, novel food procedure)	For gamma-cyclodextrin a "non-specified" ADI was established by JECFA in 1999; no gastrointestinal intolerance has been demonstrated for a consumption of up to 8 g/person/day.	
Trans-galacto-oligosaccharides (TOSs)	2001-SA-0191 (preparations for infants and young children)	Mixture of FOS-TOS (10%/90%) accepted at the amounts of 0.8 g/100 mL (infant formula) and 0.4g/100 mL (formula for young children).	
Resistant starch	2003-SA-0162	Good digestive tolerance of the ingredient in adults at maximum doses expected in the diet (< 30 g/d). Avoid in young children.	
Isomaltulose	2004-SA-0410/2004-SA-0169 (NF)	No threshold data. One study reported no digestive problems if consumption < 48 g/d.	
Arabinogalactan	2005-SA-0098	Consumption of arabinogalactans poses no risks to consumer safety. However, the risk of digestive discomfort (flatulence, bloating) associated with the consumption of arabinogalactans should be indicated on the labelling of products containing arabinogalactan.	
Dextrin	2005-SA-0283	Considered a soluble fibre, no upper limit value defined. Digestive tolerance threshold estimated at 45 g/d for healthy adults.	
D-tagatose	2005-SA-0305	Tolerance threshold 10-25 g/d	

Polydextrose	2003-SA-0232 related to 2002-SA-0183 authorised food additive E1220	Digestive tolerance thresholds ("mean laxative threshold") determined by the Scientific Committee on Food (SCF), at 90 g/d or 50 g in a single dose; with respect to digestive tolerance, diarrhoeal episodes have been reported in some volunteers who received 15 g of polydextrose per day; however, it is plausible, based on the available scientific data, that the digestive tolerance threshold of polydextrose is higher than that of FOSs for example (30 g/d), given that the latter would be much more fermentable; in addition, according to the available scientific data, the presence of flatulence after consumption of products fortified with polydextrose cannot be excluded	
Maltodextrin	2006-SA-0140 (Opinion on sugars), 2007-SA-0102	Maltodextrins (dextrose equivalent (DE) 5-20) are as hyperglycaemic as glucose or maltose. The insulin responses are dose-dependent → included in sweeteners whose consumption should be reduced. Infant formula against diarrhoea containing 68 g/100 g of maltodextrin, considered as safe	
Glucoronolactone	2006-SA-0236, 2005-SA-0111 2002-SA-0260 2000-SA-0191	Suspicion of renal toxicity for D-glucuronolactone	
Docosahexaenoic acid (DHA)	See new ANCs (2006-SA-0359)	No risk identified	
Eicosapentaenoic acid (EPA)		No risk identified	
Stearidonic acid (SDA)	2007-SA-0389 related to 2007-SA-0242 echium oil	No risk identified	
Arachidonic acid (ARA)	2005-SA-0375 (2006-SA-0201) See new ANCs (2006-SA-0359)	The use of ARA (provided by an oil from the fungus *Mortierella alpina)* in formulae for premature and term infants is widely accepted and use of this oil as a source of ARA is therefore justified	
Oleic acid/omega 9	See new ANCs (2006-SA-0359)	No risk identified	

Conjugated linoleic acid (CLA)	2002-SA-0332, 2003-SA-0388 (Report on trans fatty acids), 2006-SA-0156, 2008-SA-0176	In mice, administration of diets supplemented with 1% of CLA causes the complete disappearance of body fat, combined with hypertrophy and hepatic steatosis. The results of clinical studies showing the deleterious or beneficial effects of CLAs on various parameters are contradictory and controversial. The CLAs induce oxidative stress *in vitro* by a mechanism dependent at least partially on PPAR-α, an observation confirmed by several clinical studies.	**See this Opinion**
Lecithin	2000-SA-0181 authorised food additive E322	Considering that the product (lecithin) exhibits no particular toxicity through the data presented, but in the absence of a study conducted in a large number of subjects and with regular monitoring, the absence of toxicity in the long term cannot be established.	
Diacyl glycerophosphatidyl-choline (GPC)	2000-SA-0181	The use of diacyl glycerophosphatidylcholine (GPC) belonging to the group of lecithins in foods for particular nutritional uses; the purpose is to increase intake of GPCs, a source of polyunsaturated fatty acids and choline, and which are, moreover, the lipid components of many biological structures; in the absence of data, it is not possible to reach a conclusion on the absence of an adverse effect on a large population and in the long term from consumption of this product.	
Phytosterol/ phytostanol			**Request No. 2010-SA-0057 under way**
Amino acids and amino acid derivatives	Report on proteins (2007)		**See this Opinion**
Antioxidants: epigallocatechin gallate, flavonoids, polyphenols, carotenoids, proanthocyanidins, beta-carotene			**Deliberation under way on antioxidants**
Phytoestrogens, isoflavones, genistein	2005 Report 2008-SA-0201		**See this Opinion**

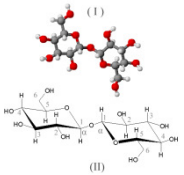
α-linoleic acid, linoleic acid, α-lipoic acid, astaxanthin, betaine, caffeine, carnitine, chitosan, chondroitin, coenzyme Q10, creatine, fructo-oligosaccharides, GABA, glucosamine, glutathione, hesperidin, inositol, inulin, L-5 hydroxytryptophan lactase, lactoferrin, lutein, lycopene, pectin, propolis, quercetin, rutin, superoxide dismutase, taurine, troxerutin, tryptophan	See Opinion 2007-SA-0231		

Annex 2. List of fibres, carbohydrates and carbohydrate derivatives that have been the subject of AFSSA Opinions

Substance	Nature and origin of the substance	Use in food and pharmacology	Formal request	Limit/information on the risk
Digestible (in the small intestine) carbohydrate polymers				
Maltodextrins (digestible)	Polymers of glucose units linked in α 1-4 and α 1-6	Frequently used as a "bulking agent", are not sweetening but provide as much energy as glucose (in fact, slightly more than glucose) or starch.	2006-SA-0140 2007-SA-0102	Maltodextrins (DE 5-20) are as hyperglycaemic as glucose or maltose. The insulin responses are dose-dependent. Included in the list of sweeteners whose consumption should be reduced (AFSSA 2007a). Infant formula against diarrhoea containing 68 g/100 g of maltodextrin has been considered as safe.
Gamma-cyclodextrin	Cyclic oligosaccharide composed of eight glucopyranose subunits linked in α-(1,4) $C_{48}H_{80}0_{40}$	Drug formulation excipient - complexation of sensitive active ingredients. Flavour enhancer, emulsion stabiliser and protection of volatile molecules. Incorporated into many liquid or solid foods at concentrations up to 8% in beverages, 10% in nutrition bars or 20% in low-fat cream spreads.	2001-SA-0119	For gamma-cyclodextrin (γ-CD) a "non-specified" ADI was established by JECFA in 2001. The compound is rapidly metabolised into glucose (dose 1g/kg live weight) in rats (WHO 2001). No gastrointestinal intolerance has been demonstrated for a consumption of up to 8 g/person/day (Koutsou *et al.* 1999; Munro *et al.* 2004). No teratogenic or genotoxic effects have been observed (doses up to 20% of food intake). Other toxicological studies cover periods ranging from 3 months to 1 year in rats; they show no toxic effect and the γ-CD was well tolerated in animals. Acute oral toxicity LD50>8000 mg/kg (rat). 90-day study in rats and dogs: NOAEL: 20% of the diet. Teratology studies in rodents: not teratogenic, embryotoxic or foetotoxic at doses ≤ 20% of the diet in rats and rabbits.
Carbohydrate polymers with the status of dietary fibre or likely to obtain it				
Arabinogalactan	Galactan (galactose polymer) with arabinan side chains (arabinose polymer) extracted from larch wood but which could be extracted from other woods and plants.	Arabinogalactan was granted the status of dietary fibre, but the "prebiotic fibre" claim was refused by AFSSA.	2005-SA-0098	Consumption of arabinogalactans (AGs) poses no risk to consumer safety. However, the risk of digestive discomfort (flatulence, bloating) associated with their consumption should be indicated on the labelling of products containing AGs. In one study, digestive discomfort (flatulence and/or bloating) was observed in approximately 3% of subjects for daily consumptions of ≥ 8.4, 15 or 30 g/d.

				The LD50 in rats is 12.9 g/kg of body weight. AG has GRAS[5] status in the US (since 2000) and received authorisation for use from the FDA as a food additive for technological purposes on the basis of the *quantum satis* principle.
Polydextrose	Glucose polymer with random links to several sorbitol terminal groups. It is obtained by melting and polycondensation of glucose in the presence of sorbitol and catalysts. Mean DP: 12	Dietary fibre. The claims concerning intestinal transit made by the applicant were rejected by AFSSA (2002-SA-0183).	2003-SA-0232 related to 2002-SA-0183	Polydextrose has been recognised as a dietary fibre. Digestive tolerance thresholds ("mean laxative threshold") were determined by the Scientific Committee on Food (SCF), at 90 g/d or 50 g in a single dose (Flood *et al.* 2004). With respect to digestive tolerance, diarrhoeal episodes have been reported in some volunteers who received 15 g of polydextrose per day; however, it is plausible, based on the available scientific data, that the digestive tolerance threshold of polydextrose is higher than that of FOSs for example (30 g/d), given that the latter would be much more fermentable; in addition, according to the available scientific data, the occurrence of flatulence after consumption of products fortified with polydextrose cannot be excluded.
Resistant starch (type 3)	Retrograded starch $(C_6H_{10}O_5)_n$, (α1-4 links, vast majority) whose most digestible fraction is eventually eliminated enzymatically	Used as dietary fibre; applications have been made for other claims (bifidogenic effect, in particular) but refused by AFSSA.	2005-SA-0285 2003-SA-0162,	Type 3 resistant starch was granted the status of dietary fibre (stimulates butyrate production in the colon). Good digestive tolerance of the ingredient in adults at maximum doses expected in the diet (< 30 g/d). Simulations predict a consumption of 5 to 9 g/d if this resistant starch is used in grain products, dairy products and milk beverages (Request No. 2003-SA-0162). Avoid in young children.
Resistant dextrin	$(C_6H_{10}O_5)_n$ α-(1,4) links ± α-(1,6) between the glucose units retrograded dextrins	Dietary fibre for which the claims made but refused by AFSSA mainly concerned a prebiotic effect	2005-SA-0283	Considered as soluble fibre, no upper limit value defined. Digestive tolerance threshold estimated at 45 g/d for healthy adults.

[5]GRAS, generally recognized as safe

Galacto-oligosaccharides (GOSs) or Trans-galacto-oligosaccharides (TOSs)	β-galacto-oligosaccharide consisting of a lactose unit and galactose units linked in β-1,6, β-1,4 or β-1,3. DP: 3-4 (mixture of DP 1 to 6). Produced from lactose by transgalactosylation.	Bifidogenic effect in infants (infant formula).	under the name of GOS 2000-SA-0332 2000-SA-0147 under the name of TOS 2001-SA-0191	A mixture of FOS-GOS (10/90) was accepted at 0.8 g/100 mL (infant formulae) and 0.4 g/100 mL (formulae for young children). The bifidogenic effect of the mixture has been shown. The long-term safety, although likely, remains to be demonstrated. Therefore, it is necessary "to remain vigilant on the possible long-term effects associated with the use of such preparations and careful monitoring is strongly recommended". (Alles *et al.* 1999; Wisker 2003; Zentek *et al.* 2002) In some publications (before 2000), GOS are α-gluco-oligosaccharides.
Monosaccharides and disaccharides not included with "sugars" (in the regulatory sense of the word)				
Trehalose	α-D-glucopyranosyl- α -D-glucopyranoside $C_{12}H_{22}0_{11}$ (α-1-1' link). Natural non-reducing disaccharide (fungi including yeast, honey) but obtained enzymatically 	Stabilisation of certain foods during drying processes (powdered milk, dried soup, etc.).	2000-SA-0250	There is a lack of information on the safety of two enzymes (maltooligosyl trehalose synthase and maltooligosyl trehalose trehalohydrolase produced by *Arthrobacter ramosus* and strains of *A. ramosus* and *Pseudomonas amyloderamosa)* used for the production of trehalose. Trehalose is normally hydrolysed to two glucose molecules by intestinal trehalase. Doses of 50 g would be fully tolerated by humans, who are able to digest it (Richards *et al.* 2002). This information must be provided to the consumer (especially for diabetics). There are people who are intolerant to trehalase because they are deficient in trehalose (intestinal) but they are less numerous than those deficient in lactase. Trehalose has been the subject of several studies (including genotoxicity and acute toxicity), in several animal species, that have demonstrated the safety of the compound in these animals. The use doses claimed by the applicant (3.9 to 8 g/day) are not admissible (problem with simulation studies and the safety margin advanced). Trehalose does not seem to present a significant risk to consumer health. The FAO and WHO (Expert Committee) did not consider it necessary to propose an ADI value for trehalose.
Isomaltulose	Isomer of sucrose with α1-6 link between glucose and fructose; $C_{12}H_{22}O_{11}$ Found in honey and	Used as a sweetener (despite a sweetening power of 0.42) in beverages and confectionery to reduce	2004-SA-0410 2004-SA-0169 (NF)	It is hydrolysed by the sucrase-isomaltase enzyme complex of the small intestine, into glucose and fructose. Digestion is slower than sucrose, but provides as much energy (van Can *et al.* 2009) => Need to inform consumers about the fact that it is a "caloric" and glycaemic sweetener.

	sugar cane; produced by biotechnology (*Protaminobacter rubrum*)	intake of sugar (and other caloric sweeteners).		Studies in healthy and diabetic subjects showed good tolerance up to 50 g per day with no sign of digestive discomfort. As formaldehyde is used to destroy *P. rubrum* bacteria, particular attention must be paid to the processes of elimination of this compound. Among the oral toxicity studies, one for 13 weeks in rats (male and female) at doses of 7.0 to 8.1 g/kg of body weight showed no signs of toxicity and good tolerance in animals (Lina *et al.* 2002).
D-tagatose	Epimer of D-fructose, detected at low concentrations in dairy products processed at high temperatures (i.e. dried and sterilised milk); synthesised from lactose by lactase from *Aspergillus oryzae*.	Low-energy carbohydrate sweetener (1.5 kcal/g, according to the applicant). It is also a humectant, texturiser and thickener and is used as a bulking agent.	2005-SA-0305	GRAS status with the FAO/WHO since 2001. Tolerance threshold 10-25 g/d. AFSSA advises against the consumption of this product in children under 3 years and calls for studies in children over 3 years. The fact that D-tagatose is produced from lactose requires consumer information on the risk of the presence of milk proteins (Directive 2003/89/EC amending Directive 2000/13/EC) unless the petitioner applies for exemption.
Glucurono-lactone	Lactone (cyclic ester) derived from glucuronic acid $C_6H_8O_6$	Used in some energy drinks because it supposedly helps in combating fatigue.	2006-SA-0236 2005-SA-0111 2002-SA-0260 2000-SA-0191	Suspicion of renal toxicity for the D-glucuronolactone - not confirmed by a 13-week study in rats. Germany < 2400 mg/L in soft drinks (German Fed. Risk Institute, March 08). NOAEL: 1000 mg/kg bw/day (rats). No studies on the genotoxic effects, teratogenicity, carcinogenicity (EFSA 2009d).