Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the use conditions for certain substances other than vitamins, minerals and plants in food supplements – 2

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Abstract

In Spain, food supplements are regulated by Royal Decree 1487/2009, which transposes into the Spanish law the Directive 2002/46/EC on the approximation of the laws of the Member States relating to food supplements. However, only the use of vitamins and minerals is currently regulated. Therefore the Scientific Committee of Spanish Agency for Food Safety and Nutrition (AESAN), in its report approved on 28 November 2012, dealt with the conditions that certain substances other than vitamins, minerals and plants should meet to be used in food supplements, and in order to be included in a new annex III of Royal Decree 1487/2009.

In view of the observations made to the proposals already assessed by the Committee in its previous report, a new proposal has been elaborated by AESAN. This new proposal includes the increase of certain maximum daily quantities or substances related with those previously assessed. The eight substances or groups of substances proposed by AESAN are: branched-chain amino acids, L-histidine, L-glutamine, ubiquinol, linoleic acid and alpha-linolenic acid, myo-inositol, quercetin and rutin.

The Scientific Committee has assessed each proposal and has stated, in each case, whether the proposal submitted by the AESAN is acceptable for its use as a food supplement, from a safety point of view. In no event is the assessment intended as a guarantee of the efficiency of the substances and the doses assessed.

Key words

Food supplements, amino acids, ubiquinol, linoleic acid, alpha-linolenic acid, myo-inositol, quercetin, rutin.

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Introduction

The Scientific Committee for the Spanish Agency for Food Safety and Nutrition (AESAN), in the report approved in its plenary session dated 28 November 2012, addressed the conditions of use of certain substances other than vitamins, minerals and plants to be used in food supplements.

Royal Decree 1487/2009, of 26 September, relating to food supplements currently only considers vitamins and minerals among the substances authorised for use in the manufacture of food supplements in Spain. The lack of regulation relating to the manufacture in Spain of food supplements containing substances other than vitamins and minerals has prevented their manufacture at national level, but not their marketing through the use of the authorisation obtained in another Member State and the corresponding mutual recognition. Therefore, AESAN has drawn up a proposal relating to certain substances other than vitamins and minerals for use in the manufacture of food supplements and their corresponding maximum daily quantities for inclusion in a new annex III of Royal Decree 1487/2009.

In the light of certain observations made regarding these proposals already assessed by the Committee by some of the independent communities and sectors involved, a new proposal has been drawn up by the AESAN, increasing certain maximum daily quantities or including substances linked to those assessed in the previous report.

Therefore, the Executive Director of the AESAN has asked the Scientific Committee to assess the proposal to authorise the use of certain substances other than vitamins and minerals in the manufacture of food supplements both with respect to the maximum daily quantities proposed and as regards the appropriateness of the authorisation.

Branched-chain amino acids (L-isoleucine + L-leucine + L-valine)

1. Proposal

The AESAN proposes a maximum daily quantity of the sum of L-isoleucine, L-leucine and L-valine of 5.45 g. This proposal is based on the sum of the maximum individual doses, previously validated for each amino acid by the Scientific Committee of the AESAN in an earlier report (AESAN, 2012), which totals 5.45 g (isoleucine (1.5 g) + leucine (3 g) + valine (1.95 g) = 5.45 g).

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) highlights that the subacute and acute toxicity studies conducted on rats using branched-chain amino acids (in the proportion 2,1:1:1,2 for L-leucine:L-isoleucine:L-valine) indicated that the mean acute lethal dose is more than 10 g of branched-chain amino acids/kg b.w. (Okazaki et al., 1989), whereas the chronic toxicity studies, on rats, indicates that doses of 2.5 g/kg b.w./day for 3 months or 1.25 g/kg b.w./day for 1 year did not have any toxic effect (Okazaki et al., 1989). In humans, the majority of the sports supplement studies have used doses of more than 5 g of total branched-chain amino acids, without detecting toxic effects (Shimomura et al., 2004). Furthermore, no adverse effects on health were observed after administering a total of 14.4 g of branched-chain amino acids, for 30 days (DeLorenzo et al., 2003). In addition, enteral administration, in doses of 240 mg/kg b.w./day for 6 months, in patients with hepatic encephalopathy, sepsis or multiple trauma, did not cause any toxicity or adverse effects (Baker, 2005).

Nevertheless, a reduction was observed in the content of L-methionine and aromatic amino acids based on a study conducted on five individuals who were administered a single acute dose of 5 g of branched-chain amino acids (in the proportions 1:2,3:1,2 for L-isoleucine:L-leucine:L-valine). In addition, a transitory increase in the plasma levels of insulin was observed, without this affecting the glycaemia or the plasma levels of free fatty acids (Zhang et al., 2011). In addition, the Scientific Committee of the Norwegian Food Safety Authority established that branched-chain amino acids present a moderate risk and changes take place in the biomarkers, but without adverse effects to health (VKM, 2011). Moreover, there is no maximum tolerable intake level, as no toxicity studies on humans are available to provide these bases.

3. Conclusion

The Scientific Committee has recently evaluated a maximum daily quantity of 5 g of the sum of L-isoleucine, L-leucine and L-valine and concluded that is acceptable from a safety viewpoint for use as a food supplement, provided that these amino acids are not consumed by pregnant women, children or for prolonged periods of time without medical supervision. The Committee also stated that, according to several studies, intake levels of up to three times more than the intake recommendations are well-tolerated by healthy adult subjects.

The Scientific Committee concluded that, based on the information available to date and taking into account the considerations indicated in its report dated 28 November 2012, the proposal of a maximum daily quantity of the sum of L-isoleucine, L-leucine and L-valine of 5.45 g is acceptable from a safety viewpoint for use as a food supplement, with the warning that these amino acids are not consumed by pregnant women, children or for prolonged periods of time without medical supervision.

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L-histidine

1. Proposal

The AESAN has recommended a maximum daily quantity of 1.12 g of L-histidine. The proposal is based on the fact that Regulation (EC) No 953/2009 (EU, 2009) includes L-histidine among the substances that may be added for specific nutritional purposes in foods for particular nutritional uses. In Belgium, L-histidine is authorised in food supplements without the establishment of a maximum daily quantity (Belgium, 1992). In Italy, a maximum daily quantity of 1.12 g of L-histidine is authorised in food supplements (legislative proposal) (Italy, 2012).

2. Safety

The report of the Scientific Committee dated 28 November 2012 (AESAN, 2012) indicated that in the case of studies on animals, the administration of L-histidine by intraperitoneal or intravenous injection caused changes in the amino acid content in the brain (Oishi et al., 1989) and histamine levels (Schwartz et al., 1972), in rats caused a reduction in locomotive activity after receiving an intraperitoneal injection of L-histidine (250 mg/kg b.w.) (Dutra-Filho et al., 1989) and "strange behaviour" was observed in rats administered with intraperitoneal L-histidine in concentrations of 400 to 800 mg/kg b.w. These effects have not been indicated in rats administered with L-histidine orally.

Low protein diets enriched with L-histidine, administered to rats, for 3 to 4 weeks, led to significant weight loss after some days. Nevertheless, the effects diminished when increasing quantities of high-quality proteins were added to the diet (Benevenga and Steele, 1984).

In studies on rats fed for short periods of time (7 to 46 days), with between 2 and 4 g/kg b.w./day of L-histidine, delayed growth, hepatomegaly and hypercholesterolemia were observed (Solomon and Geison, 1978) (Harvey et al., 1981) (Ohmura et al., 1986) (Hitomi-Ohmura et al., 1992). Harvey et al. (1981) indicated significant reductions in the plasma concentrations of copper and zinc and a reduction in the copper content in rat livers after receiving, for 46 days, diets containing 8 % of L-histidine (~4 g/kg b.w.).

The long-term toxicity and the carcinogenicity of L-histidine monohydrochloride (HMHC) was studied in 50 male rats and 50 female rats (Ikezaki et al., 1996). The male rats were administered diets containing 0.47 and 0.96 g/kg b.w./day of HMHC for 104 weeks and the females 0.56 and 1.1 g/kg b.w./day for the same period of time. No significant increases were observed in the appearance of tumours related to the treatment when compared to paired controls. Nor were any neoplastic changes mentioned in either the control groups or the treatment groups. In the male rats that received 0.96 g of HMHC/kg b.w./day increases were observed in the red blood cell count, haemoglobin and hematocrit concentration. No sperm granuloma tests were observed in male rats that received either 1.6 g of HMHC/kg b.w./day for 13 weeks or 0.97 g/kg b.w./day for 104 weeks (Ikezaki et al., 1996).

Regarding its adverse effects on humans, it was stated that no adverse effects as a consequence of the L-histidine therapy were observed (Pinals et al., 1977), although it is not clear as to what the adverse effects examined were.

Similarly, in a test carried out by Blumenkrantz et al. (1975), no adverse effects were mentioned, although it is not clear from the report which effects were examined. Studies of the effects of L-histidine on taste and smell acuity produced contradictory results. Henkin et al. (1975) indicated a reduction in

acuity in both senses in six patients who received 8 to 65 g of L-histidine/day, for 24 days. In view of the increase in the urinary excretion of zinc and the decrease in the serum level, the authors postulate that the effects of the administration of L-histidine are due to a zinc deficiency. In another study in which eight healthy men were administered with 4 g of L-histidine/day, for 2 weeks, the effects on taste and smell acuity were not mentioned (Schechter and Prakash, 1979). This also occurred with the administration of oral doses of L-histidine, of between 24 and 64 g/day, for 4 weeks. Nevertheless, even at the lowest dose (4 g/day) adverse effects were observed. These included headaches, weakness, stupor and nausea, whereas at the highest doses (24 and 64 g/day) anorexia, feeling of pain in the eyes and changes in visual acuity in two women were reported (Geliebter et al., 1981). In children who received total parenteral total nutrition an increase is mentioned of 70 % in the urinary excretion of zinc when the fluid contains 165 mg of L-histidine/kg b.w./day, compared to the 95 mg of L-histidine/kg b.w./day of the controls. Although this is parenteral administration, it provides further evidence that in humans an intake of L-histidine in excess may cause L-histidine/zinc interactions, the result of which is a zinc deficiency (Zlotkin, 1989).

With regard to the dose-response assessment, it was concluded that the available scientific data are inadequate for deriving an UL for the chronic oral intake of L-histidine from food supplements.

3. Conclusion

The Scientific Committee concludes that, based on the information available to date and taking into account the general considerations reflected in its report dated 28 November 2012, the AESAN proposal of a maximum daily quantity of 1.12 g of L-histidine is acceptable from the safety viewpoint for use as a food supplement.

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L-glutamine

1. Proposal

The AESAN has recommended a maximum daily quantity of 5 g of L-glutamine. This proposal is based on the fact that L-glutamine is authorised In Italy in food supplements (legislative proposal) without the establishment of a maximum daily quantity (Italy, 2012).

Moreover, the NDA Panel (Panel on Dietetic Products, Nutrition and Allergies) of the EFSA (European Food Safety Authority) has published two reports (EFSA, 2009, 2011) on the benefits claimed for glutamine, in which the proposed doses range between 50 and 900 mg/kg b.w./day, or even 100 mg at 5 g per portion or per day.

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) stated that there were numerous studies on the use of L-glutamine as a food supplement, but only four have been designed with the objective of assessing its safety (Garlick, 2001). From these studies it was concluded that L-glutamine is safe in adults and neonates but that there is insufficient data to permit the identification of one or more adverse effects. The studies carried out have not permitted the establishment of a NOAEL for L-glutamine (Garlick, 2001). The maximum doses used in the few studies carried out were 0.3 g/kg b.w. in a single oral dose or 0.57 g/kg b.w. administered intravenously, for 30 days. There is also a study which used between 20-40 g/day, but only for 24 hours. Nevertheless, a

series of considerations must be taken into account: i) there are no studies of any sort on the use of L-glutamine in healthy subjects over long periods of time; ii) the studies have always been conducted on patients under strict medical supervision; iii) individual susceptibility must be studied, as there is a study that demonstrates intolerance to L-glutamine in doses of 0.1-1.0 g/kg b.w./day, in cases where it is used to treat Crohn's disease (Akobeng et al., 2000); iv) there are no toxicity data for the elderly, in whom high doses of L-glutamine could obstruct the hepatic and renal processing of an increased nitrogen load; and v) L-glutamine is metabolised to glutamate and ammonia, compounds that have adverse neurological effects, and therefore studies on the possible psychological and behavioural effects are required. In addition, the Scientific Committee of the Norwegian Food Safety Authority established that L-glutamine presents a low risk as no changes take place in the biomarkers nor are there any adverse effects to health (VKM, 2011).

3. Conclusion

The Scientific Committee considers that no adverse effects have been observed in either the safety studies conducted with L-glutamine or in its use at high doses in clinical nutrition. Therefore, and although the safety of L-glutamine has not been assessed in healthy subjects or in chronic administrations, this Scientific Committee concludes that based on the information available to date and taking into account the general considerations reflected in the report from the Scientific Committee dated 28 November 2012 (AESAN, 2012), the AESAN proposal of a maximum daily quantity of 5 g of L-glutamine is acceptable from the safety point of view for use as a food supplement.

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Ubiquinol

1. Proposal

The AESAN has recommended a maximum daily quantity of 200 mg of ubiquinol. The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) highlights that ubiquinone (non-reduced form of CoQ10) is transformed to ubiquinol in the enterocyte, before being released into the lymphatic circulation. In addition, around 95 % of the circulating CoQ10 is in the form of ubiquinol. Therefore, in recent years a large quantity of dietary supplements of reduced CoQ10 (ubiquinol) have been marketed. Moreover, it has been confirmed that this reduced compound is absorbed better than ubiquinone and after its intake; the plasma levels obtained are higher than those obtained following the intake of ubiquinone in similar doses, in any of its pharmaceutical forms and for low, medium and high doses. In the case of high doses, efficiency is increased if it is administered in split doses in two intakes (Bhagavan and Chopra, 2007).

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) highlighted that the safety of ubiquinone and ubiquinol has been studied by different authors, considering that it is a molecule found in its natural form in our body. The studies conducted with CoQ10, in any of its forms are based on the methods of the Council for Responsible Nutrition (CRN) (Hathcock, 2004) that include the characteristics of the determination of a UL value from the US Food and Nutrition Board (FNB) and the modification of the observed safe level (OSL) adopted as the highest observed intake (HOI) by the FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). As the data available for the different clinical tests on humans, random and placebo controlled and of adequate size and duration, did not establish adverse effects, the OSL of the CRN and the HOI of the FAO/WHO are used instead of the NOAEL or the LOAEL to establish the UL. No systematic pattern of adverse effects with relatively high doses has been observed in patients suffering from Parkinson's (2 400 mg, 1 200 mg and 600 mg). In other studies, effects have not been observed with dose ranges of 390-100 mg/day in healthy individuals with different pathologies (Hathcock and Shao, 2006). Some studies observed the appearance of nausea, heartburn, gastric upset or related effects at doses of 600 mg in cardiac patients and 1 200 mg in Huntington and 120-180 mg in angina pectoris, in HFD and heart attack patients and 60 mg in subjects with oligospermia. Nevertheless, many of these disturbances also appeared in the placebo group and no significant differences exist. The toxicity studies on animals have permitted the establishment of an ADI of 12 mg/kg b.w./day. Nor have any genotoxic effects been observed (Hidaka et al., 2008).

CoQ10 does not present acute, subacute, chronic or reproduction or development effects at the doses proposed (Hosoe et al., 2007) (Hidaka et al., 2008). There is not enough information regarding the safety of the use of CoQ10 during pregnancy and breastfeeding.

3. Conclusion

The Scientific Committee recently assessed a maximum daily quantity of 200 mg for the coenzyme Q10 or ubiquinone concluding that the toxicological studies carried out had not revealed any adverse effects. Therefore, considering that an OSL of 1 200 mg/day has been established, the Scientific Committee concludes that, based on the information available to date and taking into account the general considerations reflected in the report dated 28 November 2012, the AESAN proposal of a maximum quantity of 200 mg/day of ubiquinol is acceptable from the safety point of view for use as a food supplement.

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Linoleic acid and alpha-linolenic acid

1. Proposal

The AESAN proposes a maximum daily quantity for alpha-linolenic acid of 2 g, with a linoleic acid/alpha-linolenic acid ratio of a maximum of 5. Linoleic acid is included among the substances controlled by Royal Decree 867/2008, of 23 May, approving the specific technical and sanitary standards regarding infant formulae and follow-on formulae (BOE, 2008). This establishes the quantities and proportion between said fatty acids (not less than 5 or more than 15). In Belgium and in Italy (legislative proposal) they are authorised in food supplements without the establishment of a maximum daily quantity (Belgium, 1992) (Italy, 2012).

Moreover, the NDA Panel of the EFSA indicated that there is no convincing evidence (insufficient studies) that a high intake of alpha-linolenic acid (ALA) has a negative effect on health. According to the EFSA Opinion (2009a), ALA is the most abundant form of omega 3 fatty acid in foods. The reference intake value for ALA is 2 g per day (EFSA, 2009a). This value is consistent with the recommended intakes of ALA, based on considerations relating to cardiovascular and neurological health, of 1 % of the total dietary energy value, corresponding to 2-3 g of ALA/day for an intake of 1 800-2 700 kcal/day. The mean intakes of ALA in different European countries range between 0.7 and 2.3 g/day or 0.4-0.8 % of the total dietary energy value.

According to the EFSA Opinion (2009b), the appropriate conditions of use for the claim "contributes to maintaining normal blood cholesterol levels" are that the food must contain at least 15 % of the reference intake of 2 g of ALA/day (≥0.3 g/100 g or 100 kcal) and the amount established for the health claim of "high content in omega 3 fatty acids" is double this quantity.

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) highlights that there is no available data for the adverse effects from a high intake of ALA and linoleic acid (LA); the majority of the data refers to an excessive intake of EPA and DHA, which are biologically more powerful than their precursor, and that an excess in their intake in the form of supplements may increase the lipid peroxidation and reduce the production of cytokines (Meydani, 2000) (Vedin et al., 2008). However, the FAO/WHO Expert Committee (FAO, 2010) indicated that high intakes of these n-3 fatty acids have not had short- or medium-term adverse effects in tests on humans, and that some individuals in populations with a high intake of shellfish, consume even higher quantities with no apparent adverse effects. Experimental studies have shown that the risk of lipid peroxidation may increase when the intake of polyunsaturated fatty acids represents more than 11 % of the total dietary energy value, in particular when the intake of tocopherol is low (Elmadfa and Kornsteiner, 2009). In addition, some studies have associated the intake of large amounts of fat, in particular LA, with a greater long-term increase in the risk of cancer. In this respect, the results of a meta-analysis did not suggest the existence of a direct relation between the intake of high quantities of linoleic acid and cancer, although, based on the data from some studies, the existence of a certain increase in the risk cannot be dismissed (Zock and Katan, 1998). The NDA Panel of the EFSA highlighted that there is no consistent evidence that the intake of ALA or of any of the n-6 polyunsaturated fatty acids have adverse effects on health (for example, in the promotion of diet-related disease). It also proposed not to establish UL (tolerable upper daily intake levels) for either ALA or for the total or any of the n-6 polyunsaturated fatty acids (EFSA, 2010).

3. Conclusion

The Scientific Committee recently assessed that a maximum quantity of 1 g/day of ALA, with an LA/ALA ratio of a maximum of 5 could be accepted, on the basis that there is insufficient scientific evidence to link a high intake of ALA with the development of adverse effects; it did not consider higher quantities but, at the same time, shared the opinion given by the NDA Panel of the EFSA with respect to not establishing maximum levels of total intake for either alpha-linolenic acid, or for the total or any of the n-6 polyunsaturated fatty acids (EFSA, 2010). Nevertheless, considering the scope of the request (maximum levels in relation to the formulation of food supplements), the Scientific Committee of the AESAN considered there are reasonable grounds to establish prudent limits which are within the reference intake value of 2 g/day for ALA established by the EFSA. Similarly and although there is no established consensus on the optimum ratio of n-6 and n-3 polyunsaturated fatty acids, we considered the maintenance of the ratio of reference intake values established by the EFSA for both fatty acids (ratio of 5 corresponding to 10 g/day for LA and 2 g/day for ALA) to be adequate.

The Scientific Committee concludes that, based on the information available to date and taking

into account the general considerations reflected in the report from the Scientific Committee dated 28 November 2012, the proposal of a maximum quantity of 2 g/day of ALA, with an LA/ALA ratio of a maximum of 5 presented by the AESAN, is acceptable from the point of view of the safety of its use as an ingredient in food supplements, clearly aimed at consumers who are well-informed about the recommended or reference intake quantities (which may come from food supplements or normal foods). The Committee considers that the daily intake of 2 g of ALA as a food supplement, added to the quantity of ALA consumed as a food ingredient, might imply an intake of the fatty acid above the reference intake value (2 g) or in the upper margin of the recommendations for ALA intake based on considerations of cardiovascular health and neurodevelopment (1 % of the total energy value of the diet, that is 2-3 g of ALA/day for intakes of 1 800-2 700 kcal/day). In addition, polyunsaturated fatty acids are susceptible to oxidation and therefore the stability of the final product must be ensured.

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Myo-inositol

1. Proposal

The AESAN has recommended a maximum daily quantity of 2 g of myo-inositol. This proposal is based on the existence of an authorisation in Italy of 2 g/day without establishing a specific form of inositol (Italy, 2012). Regulation (EC) 953/2009 (EU, 2009) includes inositol among the substances that may be added for specific nutritional purposes in foods for particular nutritional purposes and does not establish any differentiation as regards their form. Moreover, myo-inositol was the form recommended in 2003 by the Scientific Committee on Food for use in infant formulae and follow-on formulae (SCF, 2003).

2. Safety

Inositols are ubiquitous compounds, cyclic carbohydrates with a basic ring structure of six carbon atoms. At present there are nine known isomers of inositol, of which myo-inositol is the most abundant isomer, with the molecular formula $C_6H_{12}O_6$. Myo-inositol is a cellular constituent mainly of the central nervous system (CNS) of mammals, which is commonly found in the diet. In the diet, 56 % is bound to lipids (Clements and Reynertson, 1977). In humans, all the myo-inositol consumed is absorbed in the gastrointestinal tract (99.8 %). In mammals, myo-inositol is present as phosphorylated derivatives, various phosphoinositides, and in its free form. The phosphoinositides (including tetra-, penta-, hexa-phosphates) are physiologically interconvertible to less phosphorylated phosphates and are present in a range of 0.01-1.0 mM in most cells.

Anderson (1914) presented the molecular structure of myo-inositol hexaphosphate also known as phytic acid, a structure that has been confirmed by modern analytical techniques (Johnson and Tate, 1969) (Barrientos and Murthy, 1996).

Phytate, the salt form of phytic acid (inositol hexaphosphate), and myo-inositol are widely found in plants; they are a form of storage for phosphorus and minerals, and approximately 75 % of the total phosphorus is mainly present in grains or seeds (Clements and Darnell, 1980) (Raboy, 2003). Other parts of the plants, including the roots and tubers are poor in phytates (approximately 0.1 % of the total) (Phillippy et al., 2003). In addition to phytate, other inositol phosphates such as inositol pentaphosphates, inositol tetraphosphates and myo-inositol are also present in seeds, but in lower extent (< 15 %). During the germination of the seeds, phytate is hydrolysed (Tabekhia and Luh, 1980) (Beal and Mehta, 1985), and therefore phosphate together with minerals such as calcium and magnesium are made available for the germination and development of the seed, explaining its significant role in the metabolism of the plant.

In humans, myo-inositol is present as phosphorylated derivatives, various inositol phosphates and in free form. The biosynthesis of the inositol is started with the phosphatidylinositol, phosphatidylinositol phosphate and phosphatidylinositol biphosphate compounds bound to the membrane, these are cleaved by the phospholipase C to form diacylglycerol and inositol phosphates. The subsequent

enzymatic processes produce a variety of mono-, bi-, tri-, tetra-phosphate inositols, depending on the specific substrate and the enzyme involved. The end product of each pathway is inositol, which is recycled back as a component of the original phosphatidylinositol precursor (Colodny et al., 1998).

The endoplasmic calcium storage is stimulated by inositol. Inositol triphosphate is released from the cellular membrane and travels through the cytoplasm until it reaches the endoplasmic reticulum. This inositol then releases the sequestered calcium, which can go on to mediate the release of neurotransmitters in response to depolarization. Myo-inositol is considered to be the major CNS non-nitrogenous osmoregulator. In theory, an imbalance in the concentration of inositol potentially affects the development and function of numerous receptors (cholinergic, adrenergic, serotonergic, glutamatergic, histaminergic).

There is evidence that the phytases present in the plants hydrolize phytate at gastrointestinal level in humans. The phytate forms less phosphorylated inositol phosphates, predominantly inositol triphosphate and pentaphosphate and finally myo-inositol. Under normal conditions, the dietary intake of inositol is only 1 g/day.

Myo-inositol is found in low concentrations in plasma, unlike the high concentration existing in the central and peripheral nervous system, a concentration attributed to an active intracellular transport of the myo-inositol (Palmano et al., 1977). In humans, the principal hydrolysis of phytate occurs in the large intestine by microbial phytases. Different phytases show different specific requirements for hydrolysing the phosphate groups from the phytate. In humans, the degradation of phytate improves the intestinal absorption of minerals and trace elements. Applying the concentration of free inorganic phosphate and myo-inositol as a marker of the phytate hydrolysis, it is confirmed that the phytate is degraded more vigorously in animals fed with a phytase-free diet than with a diet containing active phytases (Schlemmer et al., 2001).

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) stated that studies have been carried out on animals on the effects of myo-inositol on the lipid metabolism (Yagi and Kotaki 1969) (Shepherd and Taylor, 1974) (Okazaki et al., 2006), on lung cancer (Estensen et al., 2004) (Witschi et al., 2004), on neuropathy (Nakamura et al., 1997) and on diabetic cataracts (Beyer-Mears et al., 1989), when included in the diet at 0.2 to 3 %. No adverse effects were observed in any of these studies.

In addition, with respect to studies carried out on humans it is indicated that studies of supplementation have been carried out with respect to diabetic neuropathy (Agostini et al., 2006), mood changes, depression (Levine, 1977) (Levine et al., 1995) (Cohen et al., 1997), specific premature retinopathy (Friedman et al., 2000) or on the prevention of lung cancer (Lam et al., 2006). No adverse effects were observed in any of these studies. In two studies carried out by Lam et al. (2006), the effect was assessed of myo-inositol, administered in increasing doses from 12 to 30 g/day, to 16 subjects for one month. The only adverse effects observed were slight gastrointestinal upset. The maximum daily dose without adverse effects was established at 18 g/day. In a second test, the effects of myo-inositol were assessed on 10 subjects at a dose of 18 g/day for 3 months, and no adverse effects were observed.

3. Conclusion

The Scientific Committee concludes that, based on the information available to date and taking into account the general considerations reflected in the report from the Scientific Committee dated 28 November 2012, the AESAN proposal of a maximum quantity of 2 g/day of myo-inositol is acceptable from the safety point of view for use as a food supplement.

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Quercetin

1. Proposal

The AESAN has proposed a maximum daily quantity of quercetin of 300 mg with the warning "not recommended for pregnant women". This proposal is based on the existence of an authorisation in Italy, for its use in food supplements in a maximum daily quantity of 300 mg (Italy, 2012).

In addition, a maximum daily quantity is proposed for the sum of quercetin and rutin of 600 mg with a maximum of quercetin of 300 mg.

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) indicated that the JECFA (Joint FAO/WHO Expert Committee on Food Additives) were unable to reach a decision regarding the safety of this compound due to the absence of sufficient toxicological studies (JECFA, 1977). However, subsequently the International Agency for Research on Cancer (IARC, 1999) classified this flavonoid in the group of substances without carcinogenic effect for humans (Group 3) although they did relate it with some cases of carcinogenicity in animals, toxic effects that were observed when high quantities of this flavonoid were used (Kylesova, 2011). The administration of this compound increased the incidence of intestinal and urinary bladder tumours in a study on rats, results that could not be confirmed in later studies. Similarly, it indicates that, in male rats, quercetin may produce an increase,

slight but significant, in the incidence of adenomas on renal tumours (JECFA, 1977). In the *in vitro* tests a mutagenic effect was observed in the Ames test and on human lymphocytes. However, there have been a number of *in vivo* tests, with different animal species, on which this effect was not observed. As occurs with other compounds with powerful antioxidant activity, the Committee considered that, in certain conditions of use, quercetin may induce a pro-oxidant effect, widely referenced in scientific literature. In addition, it considered that it is probable that part of the mutagenic activity detected *in vitro* is the consequence of this pro-oxidant activity which will be counteracted *in vivo* by the action of natural systems of antioxidant defence, by the metabolic transformation of quercetin to derivatives that do not present these activities (methylation), due to the low bioavailability of the free genin or the high affinity of quercetin and its derivatives to the serum proteins (albumin), which is considered a detoxifying mechanism. Quercetin did not exhibit negative effects on the development and reproduction of the animals treated. However, the International Agency for Cancer Research (IARC, 1999) described the possibility of a delay in the fetal growth of rats treated orally with quercetin. Nevertheless, other studies have described that the excessive intake of quercetin may be a factor of occasional carcinogenic risk, especially at intestinal level (Matsukawa et al., 2002) or renal level, in this case specifically associated with male rats, but not female rats (NTP, 1992). In 2000, Middleton et al. revised the activity of the flavonoids at different levels and compiled the possible negative effect *(in vitro)* of quercetin on DNA, a consequence of its action as a pro-oxidant, often generating chromosomal aberrations. However, these effects have not been linked to standard pharmacologic doses of flavonoid.

Although the main objective of the majority of clinical tests performed has been the demonstration of its efficiency in the prevention and treatment of different diseases in humans, the results indicated very good tolerability and the absence of significant adverse effects in humans (Okamoto, 2005) (Harwood et al., 2007). Other studies on the potential genotoxicity in vivo have used doses of 2 g/kg (body weight in *Wistar* rats, equivalent to 140 g in an adult male) and have not revealed any harmful effects on DNA, nor have any active metabolites been observed in the liver or bone marrow (Utesch et al., 2008).

The Scientific Committee indicated that supplementation of the diet with quantities of between 360 and 1 000 mg/day, for 28 days, had not shown any harmful effects, nor were any adverse effects observed following the administration of doses of 760 mg/day, for 3 months (Kiesewetter et al., 2000). Askari et al. (2012, 2013) conducted various studies on athletes, supplementing their diet with quercetin in order to observe its effect on their physical performance and on different aspects of their health. Of particular note among these studies is a double-blind clinical trial using 500 mg of quercetin for 8 weeks. Regardless of the specific results, it was possible to observe that there were no undesirable or collateral effects resulting from its use.

Kiesewetter et al. (2000) use a dose of 760 mg/day, and after dividing by a safety factor of 10, the AFSSA *(Agence Française de Sécurité Sanitaire des Aliments)* estimated a maximum daily dose in the form of food supplements of 75 mg quercetin (AFSSA, 2008). There are no scientific studies that guarantee the safety of its intake in the form of supplements for pregnant or nursing women or for children.

Varying quantities of quercetin are found in foods, between 284-486 mg of quercetin/kg (onion), 10-25 mg/kg (black tea) or 21-72 mg/kg (apple) (Baková and Kolesárová, 2012), although there are some food and/or medicinal plants that exceed these quantities, such as capers *(Capparis spinosa)* with 1 800 mg/kg or lovage *(Levisticum officinale)* with 1 700 mg/kg, examples of plant species with a high quercetin content (Justesen and Knuthsen, 2001). Quercetin is a compound with a very low bioavailability, barely 4 % of the quantity consumed, however the quercetin present in the plant is more easily used by the organism (Reinboth et al., 2010).

3. Conclusion

The Scientific Committee concludes that, based on the currently available information and taking into account the general considerations reflected in the report from the Scientific Committee dated 28 November 2012, at present there is insufficient information to support the safety of the AESAN proposal to increase the maximum daily quantity to 300 mg of quercetin. Specific recent studies of mutagenicity are required and, although articles are available which analyse the absence of toxicity, these studies have been conducted on different populations to those for which it is usually intended, corresponding to animals used for research (which can not always be extrapolated to humans) or carried out *in vitro*. From *in vivo* experimental data, a chronic dose-dependent nephropathy was described with an increasing incidence of tubular cell adenomas in male rats after 104 weeks of treatment with high doses of quercetin (NTP, 1992), therefore the approval of an increase in the requested dose is not advised.

With respect to the maximum daily quantity for the sum of quercetin and rutin of 600 mg, with a maximum of quercetin of 300 mg, the Scientific Committee concludes that, although some studies show a certain level of harmlessness, there is insufficient information to assess the safety of the maximum daily quantity proposed, and its extension is therefore not justified.

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Rutin

1. Proposal

The AESAN has proposed a maximum daily quantity of rutin of 600 mg with the warning "not recommended for pregnant women". This proposal has been referred to the industry.

In addition, a maximum daily quantity is proposed for the sum of quercetin and rutin of 600 mg with a maximum of quercetin of 300 mg.

2. Safety

The report from the Scientific Committee dated 28 November 2012 (AESAN, 2012) indicated that some studies suggest that the administration of rutin may induce a mutagenic effect (Yu et al., 1986). However, in later studies carried out *in vitro*, a cytotoxic effect was only observed at very high doses (800 µM) and after a long period of exposure (72 and 96 hours), which may be linked to the manifestation of a pro-oxidant activity due to the metabolic transformation of quercetin (Marcarini

et al., 2011). The assessment of carcinogenicity in hamsters, following the administration of rutin at a concentration of 10 % of the diet, for 735 days was negative (Morino et al., 1982).

The Committee also indicated that there are very few tests that validate the absence of genotoxicity of the rutin (Hardigree and Epler, 1978). Different *in vitro* tests have proven rutin to have little effect, although in some cases, at higher concentrations, genotoxic effects have been observed in hepatic cells (HTC), producing primary damage in the DNA, exclusively class I (810 µM, after 24 hours of treatment). Nevertheless, the low bioavailability of flavonoid and its high level of metabolism mean that these concentrations do not reach tissue level *in vivo* (Cristina-Marcari et al., 2011). Rutin is metabolised in the gastrointestinal tract resulting in isoquercitrin (quercetin glycosides) and quercetin. These compounds are principally absorbed in the small intestine, and the bioavailability of isoquercitrin is higher. The plasma maximums of this compound do not reach concentrations of more than 1 µmol/litre, which is far below the potentially genotoxic concentrations (Reinboth et al., 2010).

It has been confirmed that very high doses of rutin (2 x 1 250 mg/kg b.w.), administered intraperitoneally, may induce slight damage to the DNA in the bone marrow cells of *Swiss-Webster* mice, however it is unlikely that a moderate consumption of this flavonoid in the form of an oral complement, may have clastogenic effects on humans, due to the low bioavailability mentioned above (Da Silva et al., 2002).

As regards chronic toxicity, in rats, the administration of 1 % of rutin in diet for 400 days did not induce negative effects on the physiological functions, or affect the organs of the animals treated (Wilson et al., 1947). According to the AFSSA, the toxicity studies carried out on animals *in vivo* did not exhibit negative effects, establishing a LD_{50} given orally of between 9.11 and 17.00 g/kg b.w. (AFSSA, 2008). Although not directly referred to rutin, but to a derivative of the same, isoquercitrin, a product of its partial enzymatic hydrolysis through the loss of terminal rhamnose, it was confirmed that supplementation of the diet of *Wistar* rats with concentrations of 0.2; 1 and 5 % of isoquercitrin, for 13 weeks, resulted in certain alterations in the male animals treated with the highest concentration (5 %), not displaying any effects in females. A slight inhibition was observed in weight gain, probably linked to a decrease in triglyceridemia and a fall in red blood cells, the concentration of haemoglobin and the hematocrit index. A NOAEL for the derivative of the enzymatic hydrolysis of rutin of 539 mg/kg b.w./day (1 % of the diet) was established in male *Wistar* rats and 3 227 mg/kg b.w./day (5 % of the diet) in females (Hasumura et al., 2004).

It was highlighted that there were no clinical tests directed at establishing the safety of the oral use of rutin, however, from the studies performed on humans to confirm the efficiency in the prevention and treatment of different diseases, it appeared that doses of more than 500 mg/day did not induce adverse effects on the blood parameters or on the hepatic function (Boyle et al., 2000).

3. Conclusion

The Scientific Committee recently assessed a maximum daily quantity of 150 mg of rutin concluding that there are very few scientific studies available that guarantee the safety of the rutosid in humans. Nevertheless, taking into account its presence in numerous foods, its low bioavailability when administered orally and considering that it is a source of quercetin, the Scientific Committee estimated

that the daily quantity of 150 mg of rutin, equivalent to 75 mg of quercetin, is not likely to have toxic effects on humans. In accordance with the AFSSA, the Committee indicated that in the case of the use of a mixture of both substances, the total intake quercetin and rutin, must be equivalent to an intake less than or equal to the 75 mg referred to quercetin. Due to the non-existence of scientific studies that guarantee the safety of its use in special population groups, in accordance with the AESAN proposal, it was not recommended for use in pregnant or nursing women.

The Scientific Committee concludes that, based on the currently available information and taking into account the general considerations reflected in the report from the Scientific Committee dated 28 November 2012, there is insufficient information to assess the safety of the proposal of a maximum daily quantity of 600 mg of rutin.

With respect to the maximum daily quantity for the sum of quercetin and rutin of 600 mg, with a maximum of quercetin of 300 mg, the Scientific Committee concludes that, although some studies show a certain level of harmlessness, there is insufficient information to assess the safety of the maximum daily quantity proposed, and its extension is therefore not justified.

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