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Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on the safety of grass pea flour consumption

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Abstract

The grass pea (*Lathyrus sativus*) is a legume that is used in different countries in its natural state or in the form of flour, for human consumption as well as for animal feed. Despite the popularity of its consumption in the form of flour in some areas for the preparation of the traditional dish, *gachas* (porridge), its use for human consumption is currently forbidden in Spain.

The Scientific Committee has carried out a review of this issue from the point of view of the safety of the consumption of grass pea flour, especially as regards its intake in the form of *gachas*.

Toxicological studies on animals provide limited information regarding intake levels and they do not allow us to determine tolerable intake values of the β-N-oxalyl-L-α,β-diaminopropionic (β-ODAP) amino acid present in grass peas. There are differences in the metabolisation of β-ODAP between animals and humans, which hinders the estimation of safe levels of grass pea flour consumption.

The cases of neurolathyrism occurred in the context of a high and prolonged grass peas intake, in which they were the staple food of the diet. In the absence of these conditions, this disease does not occur.

The Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has concluded that, in accordance with the information available at this point, the risk of the consumption of grass pea flour for the health of the general population may be considered negligible (excluding those with metabolic difficulties in detoxifying β-ODAP) under the usual conditions of this flour in the form of *gachas* by the Spanish population. Intake of portions of flour will be considered occasional when the limit of 25 g of grass pea flour/daily portion is not exceeded, with a low content of β-ODAP amino acid, not higher than 1 % β-ODAP, and in the context of a balanced diet that includes sulphur amino acids present in foods of animal origin such as meat, fish, eggs and dairy products and of plant origin such as wholegrain cereals (especially oats) and nuts.

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Key words

Grass peas, *Lathyrus sativus*, lathyrism, L-diamino-butyric, β-N-oxalyl-L-α,β-diaminopropionic acid, ODAP.

1. Introduction

The grass pea (*Lathyrus sativus)* is a legume that is used in different geographical areas for both human consumption and animal feed (just in its natural state or in the form of flour). In Spain it has been used in a traditional way for the preparation of some culinary dishes, such as porridge. At present, its use for human consumption is prohibited (CAE, 1967) despite the fact that its flour is consumed in some areas for the preparation of porridge.

The presence in its composition of a glutamate analogue, β-N-Oxalyl-L-α,β-diaminopropionic acid, known as β-ODAP should be noted; it is a neurotoxic amino acid responsible for neurolathyrism. Neurolathyrism affects the upper motor neurons, in which we observe a clinical profile of increased muscle tone in the lower limbs and ankle and knee strain, the Babinski sign and spastic gait. The upper limbs are affected only in the most severe cases (Getahun et al., 2002b).

Neurolathyrism occurs after excessive consumption of *L. sativus* seeds or flour as a staple food for several consecutive months (Kuo et al., 2007). Some authors consider a β-ODAP content lower than 0.15 % in *L. sativus* seeds to be a safe threshold for human consumption (Abd El Moneim et al., 2001) (Getahun et al., 2002a) (Franco Jubete, 2007).

In 2009, the European Food Safety Authority (EFSA) published a compendium with botanical species that could be a health concern when consumed as a food or food supplement due to their chemical composition. In this list the species *L. sativus* is mentioned due to the presence of β-ODAP in its seeds (EFSA, 2012).

In Ethiopia, India and Bangladesh, among other countries, neurolathyrism has been an endemic disease, with periods of worsening in times of famine. In recent years there has been a reduction in the prevalence of lathyrism in India (from 14/10 000 cases in 1994 to 2.5/10 000 cases in 2005), which has been attributed to the replacement in the cultivation of varieties with a high β-ODAP content by low-content varieties for safe consumption, a lower presence of toxic seeds, the application of β-ODAP elimination methods, as well as an improvement in the economy that allows access to other foods (Giménez-Roldán and Spencer, 2016). The International Agricultural Research Centre (CICAR) of India has collaborated with national partners to develop new varieties of Lathyrus to improve yield, adaptability to unfavourable environmental conditions, and food quality in human consumption and animal feed by reducing the content of β-ODAP (Yan et al., 2006).

In January 2016, a research panel led by the Indian Council of Medical Research (ICMR) recommended lifting the ban on the sale of grass pea (Giménez-Roldán and Spencer, 2016). This proposal was supported by the CICAR and the Food Safety and Standards Authority of India (FSSAI). This Authority was of the opinion that the ban on the sale and storage of *L. sativus* varieties with low β-ODAP content could be lifted in light of the information available, and the lack of evidence of neurolathyrism cases over the last 20 years (FSSAI, 2016).

In the report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the occasional human consumption of grass pea published in 2009 (AESAN, 2009), attention was drawn to the appropriateness of applying timely management measures aimed at guaranteeing information for the consumer on the maximum portions of grass pea flour and the possibility that an

excessive consumption could cause neurolathyrism. Likewise, it advised to carry out quantitative studies to recommend the appropriate thresholds.

Given the time that has passed since the last report of the Scientific Committee was issued and given that there is the possibility that new relevant scientific information has been published, it is requested that the Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) carry out a review of the status of this issue from the point of view of the safety of consuming grass pea flour, particularly as regards its intake in the form of porridge.

2. β-ODAP content in grass pea flour in Spain

Regulation (EU) No 68/2013 (EU, 2013) on the Catalogue of feed materials, includes grass pea among the raw materials that can be used for the preparation of feed and, therefore, grass pea flours are available in the Spanish market to be used in animal feed. In particular, it refers to seeds of *L. sativus* subjected to the appropriate heat treatment.

The National Food Centre (CNA) analysed 45 samples of grass pea flour and 4 of grass pea seeds (year 2012). The majority of the samples were taken in processing establishments and retail stores in Castilla-La Mancha and corresponded to 32 different batches of flour and 2 batches of seeds. In accordance with the results obtained by spectrophotometry analysis of diaminopropanoic acid (DAP) and conversion to β-ODAP content by a conversion factor by molecular weight, the β-ODAP content in these samples ranged from 0.255 to 1.039 %, reaching this percentage in only one of the samples. It should be noted that ODAP has two isomers, alpha and beta, with beta being in the majority (95 % of the total) and the most toxic isomer (Padmajaprasad et al., 1997) (Barceloux, 2008).

The CNA subsequently analysed the content of β-ODAP in 14 samples of grass pea flour by liquid chromatography (HPLC), obtaining an average β-ODAP value of 0.456 % (CV= 22.3 %) (Burdaspal, 2012).

3. Toxicity

The toxicity of β-ODAP has been reported in human studies, animal experiments and *in vitro* studies. It is a glutamate-like compound that acts as a potent agonist of the ionotropic glutamate receptors, the non-NMDA (N-methyl-D-aspartate) type, AMPA subtype (β-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid) or QA (quisqualate), in addition to inhibiting glutamate reuptake systems in synaptosomes, all causing excitotoxicity (Yan et al., 2006). However, and despite this being the hypothesis most accepted by researchers, it has been reported that it should not be the only mechanism of toxicity involved since it does not explain the different susceptibility observed, and because it has been documented that the affinity of β-ODAP for glutamate receptors is lower than that of compounds that do not show neurotoxic effects (Kristanc y Kreft, 2016). As such, the neurotoxicity associated with β-ODAP can be partially associated with other factors including: its capacity to chelate divalent ions, the inhibition of mitochondrial complex I by the generation of reactive oxygen species in the motor cortex and the spinal cord, the inhibition of tyrosine-aminotransferase associated with the increase of certain catecholamine derivatives in the brain and/or the deficiency of

sulphur amino acids in the diet, which leads to oxidative stress and depletion of glutathione (Kristanc and Kreft, 2016).

3.1 Studies in animals

Neurolathyrism has been observed in different animal species both from the experimental point of view and due to accidental poisoning. Enneking (2011) records studies in this regard from 1833 to 2007, with a variety of effects being shown. The main results obtained from experimental studies available in the scientific literature not included in Enneking (2011) are shown in Annex I.

Animal toxicity studies indicate the existence of interspecies differences (Amba et al., 2002) and greater susceptibility of young animals (Yan et al., 2016) and of males. With regard to oral toxicity studies, Spencer et al. (1986), for example, showed the induction of oral neurolathyrism in monkeys exposed to high doses (1.1-1.4 g β-ODAP/kg b.w.) with symptoms appearing 3-10 months after the start of exposure.

Amba et al. (2002) exposed rats, for 3 months, to a diet with 50 % *Lathyrus* of low (0.086 g/100 g) and high (0.6 g/100 g) content in β-ODAP, and did not observe significant changes in motor activity, although they did in other parameters such as neuroreceptor binding, intracellular calcium elevation, etc. However, guinea pigs exposed for 3 months to a diet containing 10, 50 or 80 % of *Lathyrus* with a high content of β-ODAP (equivalent to 39-312 mg β-ODAP/day) did show motor changes in addition to other parameters. The authors indicate that these percentages were chosen to simulate pollution (10 %), malpractice by traders (50 %) or a famine in which the grass pea becomes the basic diet (80 %).

Shinomol and Muralidhara (2007) exposed male mice to a diet with 30 % of *L. sativus* or *L. sativus* that was detoxified for 4 and 12 weeks and evaluated different parameters of oxidative stress in different brain tissues. The exposure regimen was selected so that the intake of β-ODAP per day corresponded to one tenth of what is required in humans to induce typical signs of neurolathyrism. A significant increase was observed in accordance with the exposure time in the levels of lipid peroxidation, reactive oxygen species, etc. in mice exposed to *L. sativus* and not in those exposed to its detoxified variant.

La Bella et al. (1997) also conducted oral studies in male rats exposed to *L. sativus* seeds (intake of β-ODAP ~6-20 mg/day) for 8 months and did not observe changes in the motor evaluation, however they did in behavioural parameters in the open field test, which were reduced after 1 month of purification, with the rats recovering until the significant differences between groups had disappeared.

None of the studies on oral toxicity in animals resulted in No Observed Adverse Effect Level (NOAEL) data or Lowest Observed Adverse Effect Level (LOAEL) data which could be used as a starting point to establish guide levels in health (e.g., tolerable daily intake). Nor are the exposure regimens used (daily for weeks/months) comparable to the sporadic exposure associated with porridge consumption. In this regard, La Bella et al. (1993) did perform acute trials of 90 min and 24 hours in which they exposed male rats to a single subconvulsive dose of 100 mg/kg of *L. sativus* extract by gastric tube (~20 mg/animal). Only an increase in cGMP levels was observed.

3.2 Studies in humans

The toxicity data linked to the frequency of consumption of grass pea by humans come from different authors. Thus, it has been indicated that neurolathyrism occurs in humans after consumption of at least 300 g/day of *L. sativus* for at least 3 months (Streifler et al., 1977) (Shibamoto and Bjeldanes, 2009). According to Lambein et al. (2001) intake of 500 mg of β-ODAP/day for 3-5 months in severe conditions of malnutrition and physical exhaustion does not cause apparent signs of neurolathyrism. And with an intake of 1 g/day, the latency period is 2-3 months, with the threshold being established at 500 mg/day. This involves an intake of *L. sativus* seeds that is around 10 (9-12) times greater than normal, since the recommended portion of legumes is between 60-80 g/portion (several portions per week, and not all days). The symptoms appear when the consumption of grass pea constitutes more than 30 % of the daily calories over a period of 2 to 3 months (Añón et al., 2009) or 3 to 4 months (Campbell et al., 1994) (Trombetta et al., 2006) or the consumption of grass pea over periods of 2-3 months constitutes a third or half of the total dietary intake (Barceloux, 2008) (Ottesen and Magnuson, 2010).

Khandare et al. (2014) carried out a survey in 115 households in the district of Gondia in Maharashtra (India), where the grass pea is cultivated and consumed. The nutritional status of the individuals, the β-ODAP content of samples of grass pea present in the households and the incidence of neurolathyrism were evaluated. The study revealed that 61 % of the population consumed this seed, in six households in quantities greater than 25 g/day due to their low economic level. Exposure to β-ODAP was estimated in some cases to be 266 mg (around 1.064 %), much lower than the high exposures during times of famine. The nutritional status was good and no new cases of neurolathyrism were detected (but two old cases were detected in people over 50 years old). The authors concluded that the consumption of small amounts of grass pea does not cause neurolathyrism. Similarly, Singh and Rao (2013) concluded that consumption of *L. sativus* is safe if it is part of a balanced diet.

Recently, Chaurasia et al. (2018) conducted a survey in the Gazipur district of Uttar Pradesh (India) on 9 345 regular consumers of *L. sativus* or Khesari (65 % 31-65 year olds, 82 % men, 81 % farmers, 5 % illiterate). Of the 97 % of respondents who consumed Khesari as the main source of food, only three cases of post-stroke paralysis, one case of post-Guillain-Barré Syndrome limb weakness, and one case of recurrent myelitis were detected. The authors concluded that if *L. sativus* is consumed in amounts lower than those that trigger neurolathyrism (300-400 g continuously for 3-4 months, according to Khandare et al., 2015) as part of a mixed balanced diet, its nutritional value can be used optimally.

Pratap Ruda et al. (2004) showed that the metabolism of β-ODAP in humans is the reason why populations where grass pea is the only dietary source have an incidence of less than 5 % of neurolathyrism. According to these authors, neither the β-ODAP ingested in the diet nor its metabolite DAP are found in the urine or faeces of individuals who traditionally grow and consume *L. sativus* (unlike in rats and monkeys). However, they are found in blood, so they conclude that humans, unlike animals, have mechanisms of detoxification or metabolisation of β-ODAP ingested, through the intestinal biota (Pratap Ruda et al., 2004) (Kuo et al., 2007). However, these authors also point out that there may be sensitive people whose ability to detoxify β-ODAP is deficient or even absent, so

they may be more susceptible to neurolathyrism, which may explain why only a limited number of susceptible individuals have symptoms of the disease.

On the other hand, there is a lower toxic risk when mixtures of grass pea flour are consumed with more than a third of flours of other cereals, particularly those rich in sulphur amino acids (Getahun et al., 2003).

In many cases, references to the frequency of consumption are not linked to a frequency or to a specific content of the toxic compound, with it being indicated, for example, that the excessive and daily consumption of *L. sativus* over prolonged periods (longer than months) can cause spastic weakness of the lower limbs in up to 6 % of the population (Tarade et al., 2007).

In addition to the continuous consumption of grass pea, there are other risk factors that are simultaneously associated with the onset of neurolathyrism. These factors include malnutrition and deficiencies of vitamins, essential metals and some amino acids, febrile illness and episodes of diarrhoea, intense physical work, sex (it is associated with males) or stress. The disease is associated more with males than females since the daily requirements of essential amino acids (including sulphur amino acids) are lower in women than in men, with sulphur amino acid deficiency being related to greater susceptibility (Lambein et al., 2001). In addition to different dietary habits, the lower presence in women is also related to hormonal factors (Getahun et al., 2002a). In men, young men are more affected (Getahun et al., 2002a). Haque et al. (1996) carried out an epidemiological study between 1991 and 1993 on a population of 629 752 inhabitants in a district in the northeast of Bangladesh and they observed a lathyrism prevalence of 14/10 000. Among those affected, only 12.9 % were women, and of the men, only 19.3 % were older than 30 years old. Similar data were found in Ethiopia in the rural district of Estie in the north and in the Amhara region in the south, where men were more affected than women (4.8:1 and 2.3:1, respectively) and the most affected age groups were those under 20 years of age (because they require a higher caloric intake) (Getahun and Haimanot, 1998) (Getahun et al., 2002a) (Tekle Haimanot et al., 2005). Stress has recently been identified as a risk factor for developing the disease. Studies carried out on animals revealed a risk three times higher in the onset of neurological symptoms in those animals subjected to stress (Kusama-Eguchi et al., 2010).

Having a balanced diet and one with an adequate distribution of caloric intake by food groups is a key element for avoiding the eventual toxic effect of the grass pea. However, information on safety thresholds is limited and there are no baseline studies that allow us to accurately estimate what can be considered to be a safe consumption frequency based on the β-ODAP content.

4. Reduction of the β-ODAP content

There are references that indicate the existence of varieties with β-ODAP contents that are as low as 0.02 % (Abd El Moneim, 2001). However, there are environmental factors that may mean the β-ODAP content in *L. sativus* seeds in crops will be much greater than in conditions of the growth of the plant in the laboratory (Fikre et al., 2011a). Although several authors indicate that it is possible to obtain seeds with low β-ODAP content, the authorisation of feed in Spain does not include a limitation on β-ODAP content, so pressure is not exerted to cause only seeds with low contents of

β-ODAP to be produced or imported, as would be necessary for a possible authorisation for human consumption.

A possible alternative to reduce the content of β-ODAP is the processing of *L. sativus* seeds or the flour since the passing of the toxin from its beta form to the less toxic alpha form during cooking may decrease toxicity for the consumer. Different processing methods have been proposed to reduce the β-ODAP content (Tekle-Haimont et al., 1993), however no methods destroy it completely (Tarade et al., 2007).

It has been shown that the passing of the beta isomer to the alpha form depends on the time and temperature and it would not exceed 40 %. As such, the toxicity would only reduce partially during cooking (Padmajaprasad et al., 1997). The temperature and storage at a neutral pH also increase the formation of the alpha isomer (Yan et al., 2006).

Table 1 details some studies that have assessed the effect of culinary treatment on the β-ODAP content.

Table 1. Effect of culinary treatment on the β-ODAP content

Soaking	Cooking conditions	β-ODAP elimination	Observations	Reference
3 min	-	30 %	Seed	Tekle-Haimanot et al. (1993)
No	Boil - 1 hour	70 %	Seed	Barceloux (2008)
No	Boil - 1 hour Decant cooking liquid	71.5-68.7 %	Husked or whole seeds	Jha (1987)
No	Boil - 2 hours Decant cooking liquid	85 %	Seed	Jha (1987)
Several hours in hot water	Boil	70-80 %	Husked seed	Spencer and Palmer (2003)
No No No Si No No	Autoclave in dry 30 min Autoclave in dry 30 min Boil 60 min (pH=8) Boil 30 or 60 min Bake 150 ºC 60 min Bake 150 ºC 60 min	39 % 27 % 57 % 56 % 82 % 88 %	Seeds Flour Seeds Seeds Seeds Flour	Akalu et al. (1998)
12 hours	Cook and decant liquid Steam cooking, roasting or sun drying	90 %	Seed	Liener (1979)
-	Boil and decant water	30-50 %	Seed	Tekle-Haimanot et al. (2005)

Table 1 shows that soaking (preferably for 12 hours) and cooking are the most effective treatments for reducing the content of β-ODAP; this reduction increases (90 %) if the cooking liquids are removed (Liener, 1979).

Therefore, prolonged cooking decreases the β-ODAP content (boiling the seeds for 1 hour and decanting the resulting water would eliminate up to 70 % of β-ODAP) (Barceloux, 2008). The boiling of *L. sativus* for 2 hours and the decanting of cooking water would eliminate almost 85 % of the toxic amino acid and in only 1 hour of treatment significant losses of 71.5 and 68.7 % of β-ODAP were observed, depending on whether the seed was husked or is complete (Jha, 1987).

In the study by Akalu et al. (1998) they reduced the content of β-ODAP in soaked samples cooked for 30 minutes and baked (150 ºC for 60 minutes) by 57 and 82 %, respectively.

The β-ODAP content in dried seeds treated in an autoclave for 30 minutes also showed a significant reduction ($p \leq 0.05$) by 39 %, compared with that of whole raw seeds. Similarly, when cooking soaked seeds, the β-ODAP content was reduced by up to 67 %. The fermentation process was not effective in reducing the β-ODAP content. Whereas roasting and autoclaving the ground samples caused a significant reduction ($p \leq 0.05$) in the β-ODAP content of up to 30 and 50 %, respectively, compared to whole raw seeds (Akalu) et al., 1998).

Soaking husked grass pea in hot water for several hours and boiling it removes 70-80 % of the neurotoxin with the cooking water (Spencer and Palmer, 2003). Other authors estimate this loss to be 30 % when the seeds are soaked with large volumes of water for 3 minutes (Tekle-Haimanot et al., 1993).

The role of different food processing techniques to reduce toxicity has been recently reviewed (Yerra et al., 2016). Practices such as roasting, soaking before roasting, soaking before cooking processes, tamarind water treatment, germination, autoclaving and frying in oil with different processing times of 15, 25 and 45 minutes do not completely eliminate the toxicity, but do reduce it considerably.

5. Reduction of the risk of grass pea consumption

In Spain, although the human consumption of *L. sativus* in seeds or flour is forbidden, the grass pea flour porridge is a traditional dish cooked frequently in Castilla-La Mancha. In its preparation, the grass pea flour is mixed with water and oil and heated until a dough is obtained to which other ingredients can be added. In this dish, unlike others, there is no possibility of washing the seeds or eliminating the washing water; according to some authors, these factors facilitate the reduction of the β-ODAP content, since the flour is cooked, not the seed.

By contrast, in Italy, the consumption of grass pea (*Cicerchia*) is permitted and these seeds are even included on the list of traditional Italian foods (Italy, 2011). Their consumption is different since it is mainly done by cooking the seeds in water, with there being the possibility of pre-soaking the seeds, prolonged cooking and eliminating the cooking water.

Dilution of grass pea flour with other flours is an alternative for the reduction of the contents of β-ODAP added to the fact that the contribution of other nutrients can have a protective effect (Getahun et al., 2005). *L. sativus* is deficient in cysteine and methionine and the consumption of cereals rich in them (such as oats) and condiments rich in antioxidants seems to be a protective factor against the onset of neurolathyrism (Getahum et al., 2003, 2005) (Tarade et al., 2007). The better balance of the essential amino acids (Spencer and Schaumburg, 1983) (Getahun et al., 1999)

along with a lower consumption of the toxin may explain the reduced risk of paralysis that is observed with the consumption of grass pea mixed with more than 1/3 of other cereals (Getahun et al., 2005). A protective effect has also been cited in studies with chickens that received a diet of grass pea and in which methionine, although there are differences with human neurolathyrism, reduced neurological symptoms (Fikre et al., 2010). Other foods rich in methionine and cysteine that can counteract stress and prevent neurolathyrism if they are ingested in the diet along with grass pea flour are meat, fish, eggs, dairy products, etc. (Enneking, 2011) (Kusama-Eguchi et al., 2011) (Khandare et al., 2014).

Historic references also indicate a protective effect of mixing grass pea flours with other flours. Thus, in the Encyclopaedia of Plants, published in 1855 (Loudun), it said that bread made from a 50/50 mixture of grass pea and wheat seemed to have no prejudicial effect, while bread made only from grass pea caused paralysis of the legs "when it was continuously consumed". Therefore, if it is taken with cereals and 0.5 kg of grass pea is consumed, the tolerance level may be close to approximately 2 g of β-ODAP per day (Lambein, 2000).

Condiments added for the preparation of some grass pea-based recipes (garlic, onion and ginger) have a well-established antioxidant activity (Chu et al., 2002) (Getahun et al., 2002b) (Hillocks and Maruthi, 2012). Getahun et al. (1999) establish a significant association between patients affected by neurolathyrism (in an epidemic in northern Ethiopia in 1998) and the consumption of immature green seeds or mature roasted seeds consumed without animal products, spices or other additives, which could improve the nutritional balance.

A practical example of the reduction of β-ODAP by the effect of processing is the case for *Shiro* consumption in Ethiopia. Until recently in Ethiopia, there have been cases of neurolathyrism and the effect of different preparations of *L. sativus* on its toxicity has been studied. Thus, several authors concluded that the consumption of raw grass pea (*Eshet*) was associated with a high risk of suffering from the disease, while other preparations were associated with a low risk, such as *Shiro* (thick sauce: made with a grass pea flour that is used to prepare the Ethiopian dish *Shiro wot* and which is obtained as a result of peeling, washing and roasting the grass pea seed with spices and vegetables) (Getahun el al., 2002b, 2005) (Fikre et al., 2011b). The reduced risk associated with the consumption of *Shiro* can therefore be explained by the antioxidant effect of the added condiments and by the complex culinary treatment that includes soaking of the seeds that can eliminate part of the toxin (Getahun et al., 2005).

Besides the mixture with cereals and the use of condiments, the fermentation that takes place in some preparations seems to have a protective activity and reduce the risk of neurolathyrism in consumers, by reducing the content of β-ODAP and it also improves the balance of essential amino acids (Kuo et al., 1995, 2000).

Conclusions of the Scientific Committee

Toxicological studies on animals provide limited information regarding intake levels and do not allow us to determine tolerable intake values of the amino acid β-N-Oxalyl-L-α,β-diaminopropionic acid (β-ODAP) present in the grass pea. There are differences in the metabolisation of β-ODAP

between animals and humans that make it difficult to estimate safe levels of consumption of grass pea flour.

The cases of neurolathyrism have occurred in the context of a high and prolonged consumption of grass pea, in which it represented the staple food. In the absence of these conditions, this disease does not develop.

The Scientific Committee of the AECOSAN concludes that, according to the information available at this time, the health risk to the general population with regard to the consumption of grass pea flour can be considered negligible (excluding people who have metabolic trouble detoxifying β-ODAP) under the usual conditions of consumption of this flour in the form of porridge by the Spanish population. It is considered a sporadic intake of flour when it does not exceed the limit of 25 g of grass pea flour/daily portion, with reduced β-ODAP amino acid content, no higher than 1 %, and within the context of a varied diet that includes sulphur amino acids present in foods of animal origin such as meat, fish, eggs and dairy products, and of plant origin such as whole grains (especially oats) and nuts.

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Annex I. Neurolathyrism in experimental models *in vivo*

Experimental model	Toxin	Conditions of exposure	Results	Reference
Chickens	β-ODAP	Young chicken (1 day old, 35-45 g): 10-20 mg/ chicken, i.p. route. Adult chicken (25 days, 60-70 g): 10-20 mg/ chicken and 1 mg/g i.p. route.	The young chickens did show symptoms but the adults didn't on the same dose; they required a 1 mg/g dose to develop symptoms. The authors attribute it to the action of the blood-brain barrier. They also observed that a state of acidosis increases the susceptibility to the toxin.	Rao and Sarma (1967)
Young and adult rats	β-ODAP	In young rats (12 days): 1.4 mmols/kg by i.p. and slaughtered after 30 min or 2 hours. In adult rats: 5 mmols/kg by i.p. route and slaughtered 2 hours later.	In young rats seizures were observed 10 minutes after administration, an accumulation of glutamine in the brain and chronic toxicity by ammonium. No changes were observed in the levels of urea, aspartic acid and glutamic acid in the brain. The adult rats did not develop symptoms, nor changes in the levels of glutamine or ammonium in the brain. A significant concentration of β-ODAP was found in the brains of young rats, but not in adults.	Cheema et al. (1969)
Rats	β-ODAP	10-day old rats exposed by i.p. route to 1 and 2 mmols/kg.	Seizures with a quicker onset were observed with greater dosage levels.	Johnstone (1973)
Swiss albino mouse	β-ODAP	Young mice (7-8 days) exposed by i.p. route to 0.35 mg/g and slaughtered 3-5 hours after treatment.	Damage to the tissues of the central nervous system. Lesions in the retina, arcuate nucleus and area postrema.	Onley et al. (1976)
Mice	β-ODAP	By i.p. route: In young mice (3 days old): 1.14-2.84 µmol/g . In adults: 4.26-13.3 µmol/g. By oral route: 100 mg per animal.	Severe neurological signs after a dose of 7.67 µmol/g b.w. (i.p.) or 21.3 µmol/g b.w. orally. Greater susceptibility of young mice. The LD_{50} (i.p.) for young mice was 1.71 µmol/g b.w. and in adults it was 21.3.	Metha et al. (1979)
Male Wistar rat	α and β-ODAP	Intrathecal injection of 75 µg. β-ODAP or 75-1 130 µg β-ODAP, 4-6 injections, 2 times/week. Slaughtered 2 weeks after the last injection.	The α isomer did not show acute or chronic toxicity. The β isomer displayed both. They showed a permanent bilateral spastic extension of the hind legs. The histopathology displayed notable changes in the grey and white matter of the lumbar segments, degeneration of neurons, axonal damage, etc.	Chase et al. (1985)

Experimental model	Toxin	Conditions of exposure	Results	Reference
Wistar rats	β-ODAP	Experiment 1: Newborns received a 400 mg/kg subcutaneous injection into the skin of the back or laterally. They were sacrificed 5, 15 and 60 min after the treatment. Experiment 2: Male newborns were injected repeatedly over 6 days with 200 mg/kg and they were fed with their mothers for 1 month. They were slaughtered after 13 weeks of treatment.	Experiment 1: The concentration of β-ODAP was very low in the central nervous system compared to the serum. It was not found in the cerebral cortex, diencephalon or cerebellum but it was found in the spine, mainly in the caudal region. Experiment 2: After repeated exposure, around 35 % of the animals died, 3.26 % developed irreversible paralysis, 4.34 % reversible paralysis and 57.60 % showed no signs.	Kusama-Eguchi et al. (2005)
Male Wistar rats	Synthesised β-ODAP Extract of *L. sativus* seeds	By i.p. route: 10-100 mg/kg extract and slaughter 30-180 min later. By gastric tube: 1 dose of 100 mg/kg extract and slaughter between 90 min and 24 hours later. By i.p. route: 10-100 mg/kg synthetic toxin and sacrifice 90 min later.	There were no changes in behaviour or at the motor level, since the doses used were 80 times lower than those needed to cause seizures. An increase in cGMP levels in the cerebellum after i.p. administration was observed both of toxin and extract. Oral administration also increased cGMP.	La Bella et al. (1993)
Male Wistar rats	*L. sativus* seeds	Group I. Standard diet. Group II. *Cicer arietinum* (non-toxic legume) seeds. Group III. *L. sativus* seeds. β-ODAP intake was approximately 6-20 mg/day. Exposure for 8 months, clinical exam after 4 and 8 months. After exposure, 1 month of purification and slaughter.	No significant differences were observed in the motor evaluation of the different groups, neither after 4 or 8 months of exposure nor after the purification. Only the group exposed to *L. sativus* had significant changes in the behavioural parameters in the open field test after 4 and 8 months of exposure, which were reduced after purification, with non-significant values being recovered between groups.	La Bella et al. (1997)