Safety Assessment of BIOGABA®

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Novel Food Classification: 2.1.

Introduction

An application for the authorisation of a novel food containing gamma-amino butyric acid (trade name BIOGABA®) was submitted to the Food Safety Authority of Ireland (FSAI) by Giuliani SpA in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on September 29th, 2010.

BIOGABA® is a novel food produced through a *Lactobacillus plantarum* fermentation of grape must. Gamma-amino butyric acid (GABA) can be present in the final product at up to 890 mg/100 g dry weight. GABA is the primary inhibitory neurotransmitter in the mammalian central nervous system and though in chemical terms it is considered an amino acid, it is non-essential and is not incorporated into proteins.

GABA is naturally present in some foods such as certain cheeses, and in a number of non-EU countries may be added to certain foodstuffs. A number of food supplements on the EU market containing GABA claim that it reduces stress and has a calming effect. However, the primary purpose of adding BIOGABA® to foods is for its putative health benefits as a scavenger of free radicals.

The applicant proposes that BIOGABA® will be marketed in a range of food supplements, beverages and bakery products at levels that would not exceed 13.5 g/day, which is approximately equivalent to 120 mg of GABA/day. The applicant suggests that products for the final consumer would carry labels advising against consumption by vulnerable groups such as pregnant women or children less than three years old.

Following discussion with the applicant, BIOGABA® is considered to fall into the category of "Foods and food ingredients consisting of or isolated from microorganisms, fungi or algae" as per *Article 1.2(d*) of the novel food Regulation. The application dossier was prepared pursuant to Commission Recommendation 97/618/EC and in order to assess wholesomeness, BIOGABA® is considered in Class 2.1.; "Complex Novel Food from non-GM sources - the source of the novel food has a history of use in the Community".

I. Specification of the novel food

The novel food comprises a mixture of lysed *Lactobaccilus plantarum* cells, brewer's yeast extract and grape must. This is produced by a *Lactobacillus plantarum* C 48 fermentation of grape must during which GABA content can reach levels up to 890 mg/100 g dry weight. For labelling purposes the product will be named *Lactobacillus*/grape must ferment amino-butyric acid, while BIOGABA® will be the trade name. The final product will be made available in a lyophilised or spray-dried format. The novel food specification provided by the applicant would benefit from the inclusion of limits on heavy metals such as lead, mercury and cadmium.

II. Effect of the production process applied to the novel food

BIOGABA® is produced by a *Lactobacillus plantarum* fermentation process using grape must as a nutrient source. Following cell death by lysis, the entire mixture may be lyophilised or spray-dried, resulting in a GABA concentration of approximately 890 mg per 100g dry weight. The production process is not likely to alter the integrity or quality of the final novel food.

III. History of the organism used as the source of the novel food

Grape must has been predominantly used as a nutrient source in the wine making industry for a considerable period of time both in EU and non-EU countries world-wide. The *Lactobacillus plantarum* strain used was isolated from an Italian cheese and this species has a considerable history of use in the preservation of a variety of foodstuffs and in the fermentation of particular foods, particularly dairy-based products including cheese and yoghurts. *L. plantarum* was also granted Qualified Presumption of Safety status by EFSA in 2007.

IV. – VIII.

BIOGABA® is produced from non-GM grape must and the *Lactobacillus plantarum* is also non-GM, therefore these sections are not applicable.

IX. Anticipated intake/extent of use of the novel food

The applicant intends that BIOGABA® will be consumed either with a meal or throughout the day, with levels of GABA at 10 mg/dose (food supplements), 60 mg/l (drinks) and 10-50 mg/100 g (bakery products). The combination of these GABA concentrations, along with advisory labelling is reasonably considered by the applicant to be sufficient to ensure that consumption levels will not exceed 120 mg/day of GABA, which is approximately five times that estimated to be consumed daily from the normal diet.

X. Information from previous human exposure to the novel food or its source

GABA is naturally present at different levels in a number of commonly consumed foods such as bread, tomatoes, potatoes and melons, as well as fermented dairy based

products including various cheeses and yoghurts. The applicant claims that certain foods are enriched with GABA by various processes, though it is not clear which if any of these enriched foods are marketed or consumed within the EU. GABA use as a flavouring substance was evaluated by JECFA with no safety concerns identified at the levels specified. The applicant calculates a current theoretical intake of naturally occurring GABA in a balanced diet to be approximately 24 mg/day, which is approximately one fifth of the proposed BIOGABA® upper intake levels of 120 mg/day. The source of BIOGABA® is a combination of grape must and the bacterium *L. plantarum*, both of which have a long and safe history in food and beverage production within the EU.

XI. Nutritional information on the novel food

BIOGABA® is intended primarily as a nutritional support for people wishing to counter free radicals, a claim which is not addressed in this assessment. Considering the nutrient profile of BIOGABA® it is likely that consumption through food supplements, beverages, bakery products or a combination of those is unlikely to be of nutritional significance.

XII. Microbiological information on the novel food

Lactobacillus plantarum is killed by the lytic process. In the unlikely event of there being any surviving microorganisms, proliferation would not be anticipated due to the low moisture content of the final product. The satisfactory microbiological status of the novel food is supported by three separate batch test results provided.

XIII. Toxicological information on the novel food

The toxicological data submitted by the applicant on BIOGABA® were limited to a 13 week study in rabbits. Toxicological data on other foods containing GABA were of limited use as they were relatively short in duration and generally involved daily GABA intakes of less than the maximum 120 mg/day proposed for BIOGABA®. However, a number of animal studies using either GABA alone (at doses up to 500 mg/kg bw/day for up to 100 days) or food products containing GABA have not identified any adverse effects. The argument is made by the applicant that in depth studies examining genotoxicity, reproductive, developmental and chronic toxicity or carcinogenicity are not necessary for three main reasons; the current knowledge of GABA and its effects on the human biological system; the history of safe consumption through food supplements; and its natural occurrence in a number of foods and food ingredients.

Absorption, Distribution, Metabolism and Excretion (ADME)

The majority of the components that make up BIOGABA® are expected to be routinely absorbed in the body and enter normal biochemical pathways. However, data on the ADME of BIOGABA® is not presented in the dossier, while only limited

information on the ADME of orally administered GABA is presented. Despite the demonstration of a specific GABA transporter in the rat jejunum, the available data from rodent studies and studies in other species suggest a very low level of absorption and bioavailability of GABA from the intestines. In a 13-week rabbit study, measurement of plasma levels of GABA on weeks 1 and 13 showed levels below the LOQ of 30.03 ng/mL, and only isolated results where levels were slightly above the LOQ. The authors of the study concluded that no significant absorption of GABA occurred after daily administration over a 13 week period. Given the importance of GABA as a neurotransmitter, metabolism of the molecule has been extensively studied. One of the primary metabolic products is gamma-hydroxybutyrate (GHB), which has pharmacological effects, including antidepressant in the CNS. The applicant provided additional information in an overview of the toxicokinetics, pharmacological effects and toxicity of GHB. The applicant estimated that potential levels of GHB resulting from the use of BIOGABA® as a novel food would be low (approximately 0.2 mg/day, or 0.0027 mg/kg/day) and therefore not a safety concern. GABA itself has been reported by a number of authors as being substantially excluded from the CNS by the blood-brain barrier. It has been suggested that blood-brain barrier permeability of GABA can vary between species, possibly being more restricted in higher order mammals (including humans) than in rodents.

Toxicity

(1) Studies in animals on BIOGABA and other fermented GABA sources

The only toxicological study presented by the applicant included gavage administration of BIOGABA® to male and female New Zealand white rabbits. A NOAEL of 13.5 g/day/animal of BIOGABA® (equivalent to 120 mg/day/animal of GABA) was deduced for male and female rabbits from a four week preliminary toxicity study and used as the high dose level in the subsequent 13 week rabbit study. In the 13 week oral toxicity study, two male and two female rabbits receiving BIOGABA® died unexpectedly before the completion of the study. However, there were no apparent signs of toxicity in these animals at any stage before their death, which autopsy investigations suggest were more likely to be dosing-related rather than due to any ill-effects of the test material itself. A reduction in food consumption observed in rabbits given BIOGABA® was attributed to the high nutritional content of the test material. Statistically significant differences were observed in the absolute and/or relative thyroid and liver weights of females, and in the relative weights of adrenals and kidneys of males, all of which were treated with BIOGABA®. However, the applicant concluded that these differences were not treatment related.

A number of animal studies using either GABA alone (at doses up to 500 mg/kg bw/day for up to 100 days) or food products containing GABA have not shown adverse effects.

(2) Human Studies

A number of studies are cited where GABA was orally administered to men and women to determine its effect on plasma levels of human growth hormone and hypothalamic functions. Minor transient physical discomfort was recorded by some of the test subjects upon oral administration of 5 g of GABA. Rapid increases in plasma growth hormone levels were observed, peaking at up to five-fold that of normal levels approximately three hours after GABA administration and returning to normal levels after six hours. The mechanism by which levels of growth hormone are positively affected by oral administration of GABA is not yet clear, but it is apparent that a relatively large dose (>3 g; 34 mg/kg bw) is required. The reduced growth hormone release associated with a dose of 18 g of GABA suggests some form of feedback regulation. The US EPA reported that daily oral doses of 8 mM GABA/kg bw (0.8 g/kg bw) have been administered to humans for a year or more with no indication of chronic or cumulative toxicity, however the EPA study was not available for assessment by the FSAI.

(3) Allergenicity

The applicant states that none of the components of BIOGABA® are included in the list of allergens that require specific labelling. The legislation quoted is Directive 2003/89/EC that amended Directive 2000/13/EC, but this was subsequently amended by Directive 2007/68/EC. During the process of wine making most of the solid grape material (pomace) that includes skin, seeds, stems etc is removed and subsequently used as animal fodder or fertiliser. However, the BIOGABA® production process does not appear to remove solid/particulate material with the result that the final product contains proteinaceous material almost at 30% w/w. Grapes and grape products are known to have allergenic effects on certain individuals, though grapes are not on the list of allergenic ingredients requiring specific labelling in the EU and therefore are not likely to pose a significant allergy risk.

The applicant provided additional information, including a certificate of analysis showing that the SO_2 content in the final BIOGABA® product is 330 mg/kg. The applicant calculates that, considering the maximum daily intake proposed for BIOGABA® (13.5 g), this corresponds to an intake of 4.45 mg SO_2 per day. The applicant considers this to be relatively low compared to the limits for SO_2 in a portion of wine (30 mg/150 mL - Annex IB of Regulation EC No 606/2009) and the ADI of 0-0.7 mg/kg bw set by the EU Scientific Committee for Food in 1994 which means a person with an average weight of 60 kg should not consume more than 42 mg/day of SO_2.

Conclusions

The nutrient profile of BIOGABA® indicates that consumption of this product, particularly at the levels proposed by the applicant will not have a significant nutritional impact on the final consumer. The use of *Lactobacillus plantarum* in the production of BIOGABA® does not raise any immediate safety concerns as it is routinely used in the food industry, and the particular strain used was isolated from a

cheese. There is no significant concern for the allergenic potential of grape must along with the yeast extract, which together make up a significant proportion of the proteinaceous content of BIOGABA®. Additional information provided by the applicant indicates that residual SO2 content should not pose a significant risk for individuals sensitive to sulphites.

The NOAEL reported by the applicant for the 13 week rabbit study was 13.5 g BIOGABA®/animal/day, corresponding to 120 mg/animal/day of GABA. It should be noted that the intake of GABA in this study is identical to that arising from the maximum daily use level recommended by the applicant, although when this is converted to a "per kg body weight" the intake in the rabbit study was 40 mg/kg bw/day in males and 37 mg/kg bw/day in females, whereas the intake in humans, assuming a body weight of 60 kg, would be 2 mg/kg bw/day, providing a margin of safety of approximately 20. It can be argued that the study was of relatively short duration (noting the absence of any information on long-term toxicity) and that rodents would have been a more suitable test subject. In addition, the NOAEL is dependent on the assumption that the reported changes in organ weight were not treatment related

The applicant provided further results of toxicological studies in animals along with clinical studies in humans with either GABA itself or GABA-enriched foods in support of the safety of BIOGABA®/GABA. While significant adverse effects were not identified, these studies were of comparatively short duration and thus of limited use. Human studies have shown that consumption of relatively large quantities of GABA (>3 g; 34 mg/kg bw) results in 3-5 fold transient increases in plasma levels of human growth hormone, a characteristic exploited by body-builders. The GABA intake levels required by body builders are substantially greater than the 120 mg GABA/person/day intake proposed for BIOGABA®, and are unlikely to be achieved even if naturally occurring GABA-containing foods and BIOGABA®-containing foods were consumed in combination. The applicant makes the argument that a history of low level consumption, lack of absorption and known biological effects of GABA are sufficient reasons why a full set of toxicological studies are not required.

Recommendation

The FSAI has not identified any definitive safety concerns that could arise from the consumption of foods or beverages containing BIOGABA® at the proposed levels. The arguments put forward by the applicant regarding a history of low level consumption, lack of absorption and known biological effects of GABA are noted. However, the toxicological information submitted in support of this application could be considered limited for the purposes of meeting the criteria for novel food set out in *Article 3.1.* of the novel food Regulation. For that reason, the FSAI recommends that this application be forwarded to EFSA for additional consideration of the toxicology data.