

Safety Assessment of Rapeseed Protein (Isolexx™)

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Novel Food Classification: 2.1.

Introduction

An application for the authorisation of rapeseed protein (Isolexx™) was submitted to the Food Safety Authority of Ireland (FSAI) by Helm AG in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on June 25th, 2012.

Rapeseed oil is extracted from the seeds of several rape varieties (*Brassica napus*, *B. rapa*, *B. juncea* and *B. tournefortii*) and has a long history of various uses worldwide. However, the use of rapeseed oil in food and feed was restricted initially due to the presence of glucosinolates that render it unpalatable, and erucic acid, a monounsaturated omega-9 fatty acid which was found to pose a potential health risk. A new breed of rapeseed (canola), developed in the 1970s by means of conventional breeding contains very low levels of erucic acid and glucosinolates which makes the oil more suitable for food and feed use. Rapeseed oil has a history of consumption in the EU prior to 1997. The seed protein remaining after oil extraction is increasingly being used as an animal feed supplement. However, it has not been exploited as a human food source until relatively recently.

The novel ingredient (brand name: Isolexx) is a rapeseed protein from *Brassica napus* and *Brassica rapa* which is extracted from the pressed seed material (press cake) that remains after the oil has been removed. The final ingredient is primarily composed of soluble protein ($\geq 85\%$), with insoluble protein, moisture, carbohydrate, fat and ash accounting for the majority of the remaining constituents. Isolexx is to be added to the same range of foods to which soya protein is currently added and at similar levels, except where soya protein is explicitly specified such as in infant formula.

The applicant classifies Isolexx as novel in line with *Article 1(2) (e)* of the novel food Regulation EC No 258/97: (foods and food ingredients consisting of or isolated from plants,...). For the purposes of the safety assessment, Isolexx is in Class 2.1 as per Commission Recommendation 97/618/EC: (complex novel food from non-GMO sources – the source of the novel food has a history of food use in the Community).

I. Specification of the novel food

The applicant clarified that Isolexx is derived from *Brassica napus* and *Brassica rapa* only, and describes the novel ingredient as a white to off-white powder with a mild aroma and flavour, of which more than 90% passes a US 80 mesh. The novel ingredient has a specified protein content of at least 90%, with a minimum soluble

protein content of at least 85%. Erucic acid is not detectable in Isolexx while glucosinolates are generally at or below the limit of detection and total phytates are present at $\leq 1.5\%$. The specifications are underlined by four batch analyses which also include microbial analysis and additional data that clarified the status of yeast and moulds.

II. Production process

Rapeseed oil is released from the seeds of *Brassica napus* and *Brassica rapa* by mechanical pressing. The remaining seed material (press cake) is milled to reduce the particle size and subjected to solvent (butane) extraction to remove any residual oil. After solvent extraction, the seed material is resuspended in water to which phytase is added in order to degrade phytic acid, an antinutrient that can chelate certain minerals thereby reducing their bioavailability. After further standard purification procedures to remove residual solvent, insoluble proteins and carbohydrate, the novel ingredient is spray dried to leave a white to off-white powder.

III. History of the source organism

The *Brassica* family of plants that includes cauliflower, broccoli, Brussels sprouts and cabbage also includes plants more commonly known as rape, the seeds of which (rapeseed) contain significant amounts of oil, also known as rapeseed oil. Mustard is a food allergen that must be declared on the packaging when used in food and is derived from *B. juncea* or *B. nigra* from which brown and black mustard respectively are obtained. However, the specific rapeseed varieties used to produce Isolexx are *Brassica napus* and *B. rapa*. Rapeseed oil was initially used as a lubricant for engines and also as a fuel oil for cooking and lighting, with limited food and feed uses due to the bitter and unpalatable taste caused by particular glucosinolates and their metabolites. The high level of erucic acid, a monounsaturated omega-9 fatty acid associated with potentially negative health effects further reduced the appeal of rapeseed oil to the food and feed industry.

A new type of rapeseed (canola) was developed in the 1970s by means of conventional breeding, which contained very low levels of erucic acid and glucosinolates that made the seed oil more suitable for food and feed use. However, while rapeseed oil has a history of consumption in the EU prior to 1997, the seed protein that remains following oil extraction has not been exploited as a nutritional food source until relatively recently.

IV. – VIII. GM Aspects

Food and feed ingredients from a number of GM oilseed rape varieties are permitted on the EU market. In addition, a significant proportion of the canola grown around the world is genetically engineered to possess tolerance to particular herbicides. However, the applicant intends to use only non-GM varieties of oilseed rape (*Brassica napus* and *Brassica rapa*) as a source of Isolexx.

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

Isolexx will be marketed to compete with and, to an extent replace soya as a source of vegetable protein in products such as meal replacements, protein drinks, nutrition bars, soups and soup mixes, breakfast cereals and meat analogues. The novel ingredient would also be used as a means of improving the texture of certain bakery products, chilled or frozen processed meat products, pasta and desserts.

Rather than use more recent EU-based food consumption data, the applicant provides hypothetical intake estimates for the novel ingredient in the EU, as derived using 2008/2009 Euromonitor reports and proposed soya protein isolate intakes in the US. On this basis, the average intake of Isolexx in Europe would be 1g/person/day, with intake at the 90th percentile of approximately 2-3g/day. The applicant presents a 'worst-case' scenario based on a 2011 EFSA opinion on dietary reference values for protein. Using the EFSA population reference intake (PRI) of 0.83 g/kg bw/d, the daily intake of the novel ingredient by the average (~70Kg) adult was calculated to be ≤10g/d, where 18% (0.15g/kg bw/d) of the daily protein intake came from protein added to processed food, only some of which would be made up by Isolexx. To date, no tolerable upper level has been developed for protein by EFSA and intakes of twice the PRI (~1.5 g/kg bw/d) have been considered safe.

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

Rapeseed oil has a considerable history of safe food use worldwide. However, protein from the seed material that remains after the removal of the oil is only recently being examined as an alternative source of vegetable protein in food for reasons already discussed in section III. The applicant cites FDA GRAS notices under which two distinct rapeseed proteins may be placed on the US market since 2010, and Isolexx since 2011.

XI. Nutritional information on the novel food

The major nutritional component of Isolexx is protein, constituting more than 90% in terms of dry matter. With the possible exception of lysine and the sum of the sulphur containing amino acids (cysteine and methionine), the applicant demonstrates that Isolexx compares favourably in terms of essential and non-essential amino acid content and digestibility with soya protein, the predominant vegetable protein currently added to food. Therefore, it is unlikely that there will be any nutritional disadvantage associated with the substitution of soya protein by Isolexx. It may be prudent however, to ensure that there is a balance in the amino acids for those population groups with potentially high intakes such as young active males.

Some variability in mineral content was evident between batches of the novel ingredient, particularly sodium levels, though such variations were even more pronounced in foodstuffs containing soya protein. However, vegetable protein is not a significant source of minerals in the average human diet and therefore any variation will not have a significant nutritional impact. While a positive comparison of the

novel ingredient with soy protein does not constitute a safety assessment in itself, it does provide a level of reassurance that the novel ingredient is of similar nutritional value to the predominant vegetable protein currently on the market and which it is intended to at least partially replace.

XII. Microbiological information on the novel food

The specifications relating to microbial content are satisfactory and substantiated by batch analysis results, including additional data provided by the applicant clarifying the status of yeast and moulds.

XIII. Toxicological information on the novel food

The applicant bases the safety of Isolexx on the fact that its protein content, which accounts for more than 90% of the ingredient, has an amino acid profile similar to soya protein which in turn has a considerable history of safe food use. In addition, any undesirable substances naturally occurring in rapeseed are either absent or at such low levels as to be considered not to pose a safety risk in the context of the intended uses. A number of toxicological studies on rapeseed protein isolates (not Isolexx) are referenced. These include a 90-day study in rats and dogs carried out in 1976, a 28-day study in rats from 1993, two more recent 90-day studies in rats on Puratein™ and Supertein™, the globulin and albumin storage proteins respectively in rapeseed protein and a study examining parameters of female reproductive performance and teratogenicity in rats and mice. None of the studies identified any cause for concern, although the two 90-day studies on Puratein™ and Supertein™ showed weak evidence of an anti-thyroid effect at high intake levels (up to 20% in the diet, or greater than 11 g/kg bw/day) which would not be unexpected considering the presence of glucosinolates or their metabolites in those proteins. A number of feeding trials have also been carried out with rapeseed meal in target species without adverse effects while rapeseed and by-products of the rapeseed oil manufacturing industry are positively listed in the EU as feed materials. In the absence of specific data on the toxicological safety of the protein component of Isolexx, the comparative approach is considered reasonable in view of the fact that vegetable protein from soya or rapeseed will be similarly metabolised.

Allergenicity

Mustard is a known food allergen and is one of the 14 different food allergens that, under EU legislation, must be declared when it is used in food production. Some members of the *Brassica* family are also known as mustard plants and produce seeds that are used as spices in cooking or to prepare mustard as a condiment. While “mustard” as a food allergen is not specifically defined in EU legislation, the applicant clarified that only *Brassica napus* and *Brassica rapa* would be used as the source of Isolexx, but not those plants used in the production of brown (*Brassica juncea*) and black (*Brassica nigra*) mustard. These “mustard” *Brassica* members are identified in the Codex standard relating to named vegetable oils (*CODEX STAN 210-1999*) which defines mustard seed oil: “2.1.7 **Mustardseed oil** is derived from the seeds

of white mustard (*Sinapis alba* L. or *Brassica hirta* Moench), brown and yellow mustard (*Brassica juncea* (L.) Czernajew and Cossen) and of black mustard (*Brassica nigra* (L.) Koch).”

The EFSA NDA panel in a 2004 opinion on the evaluation of food allergens for labelling purposes listed *B. nigra* and *B. juncea* as sources of mustard, while the 2010 EU Common Catalogue of Varieties of Agricultural Plant Species (<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2010:337A:0001:0660:EN:PDF>) confirms this association. The applicant also provided some scientific evidence that shows that there is little if any cross-sensitivity between the mustard and non-mustard *Brassic*as in terms of allergenicity.

With this clarification of the plants from which “mustard” is derived, along with the evidence for a lack of cross reactivity with other non-mustard *Brassica* family members, it is reasonable to conclude that foods containing Isolexx would not need to declare the presence of mustard under food allergen labelling requirements as set out in EU legislation.

Undesirable secondary plant metabolites

The possible presence in Isolexx of secondary plant metabolites including glucosinolates, allyl isothiocyanate (AITC) derived from glucosinolates, phenolics (sinapine), phytates and erucic acid are all addressed by the applicant. Data is provided showing that these residues are either not detected, as is the case for AITC, or are controlled at low levels and therefore their presence in Isolexx at the levels reported are not of toxicological concern.

Erucic acid

Erucic acid is not detectable in the novel ingredient and is therefore not of toxicological concern.

Glucosinolates

Glucosinolates occur naturally in cruciferous vegetables and along with their metabolites account for the characteristic odour and flavour. High levels of glucosinolates or their metabolites in the diet can affect the activity of the thyroid gland. However, glucosinolates are generally undetectable in Isolexx, with a limit of detection of 0.1mmol/kg (40–50 mg/kg). In some instances a batch may contain glucosinolates at levels ≤ 0.2 mmol/kg. The applicant estimates that a daily intake of 20 g of the novel ingredient would result in a glucosinolates intake of approximately 4 μ mol (~2 mg), which is equivalent to 0.5 g boiled Brussels sprouts or 2.5 g cooked cauliflower and therefore does not pose a significant safety concern.

Phytic acid

Phytic acid, the major storage form of phosphorous in plants is not digestible by humans. If ingested in sufficient quantities, phytic acid can restrict the bioavailability of certain minerals, but with a specifications limit of $\leq 1.5\%$, similar to that of soya

protein isolates, the applicant concludes that the novel ingredient does not pose a significant risk in this regard.

Conclusions

Rapeseed represents a relatively new source of vegetable protein in the EU. Rapeseed meal is established as an animal feed supplement in the EU, but only rapeseed oil has a history of consumption in food. A number of rapeseed protein isolates have already been approved for food use in the US, but rapeseed as a source of protein for food use in the EU has not been authorised yet.

Isolexx is intended as a protein source to be added to food, with only minor levels of carbohydrate, fat, ash moisture, minerals and fibre present. Protein is metabolised in the body to small peptides and individual amino acids in the gastrointestinal tract and this process does not vary significantly in relation to the protein source. Therefore it is reasonable to assume that purified protein from different plant sources with similar amino acid profiles would have approximately the same nutritional value. From a nutritional perspective it would have been preferable to rely more on recent food consumption data relevant to the EU rather than the extrapolation of US data. However, with the possible exception of high protein food preparations for use by very active individuals, the replacement of soya protein by Isolexx would not be expected to have any significant nutritional impact.

Protein in itself is not a toxic material when ingested at normal dietary levels and the applicant has argued that there is no need for a full range of toxicological studies. The applicant has chosen to compare the protein component of Isolexx to soya protein which is the predominant vegetable protein currently added to foods and has a long history of safe use. The applicant has addressed the undesirable substances that initially prevented rapeseed from being used in food or animal feed and concluded that their absence or relatively low level presence in Isolexx means that they do not pose a safety risk.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns with the consumption of foods containing the rapeseed protein Isolexx which is intended to be added in the same range of foods (except infant and follow-on formulae) and at the same levels that soya protein is currently added. The FSAI therefore considers that this novel ingredient meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation.

The applicant argues that foods containing Isolexx should not be required to declare the presence of mustard as an allergen. Despite the lack of a precise definition of mustard in EU food allergen labelling legislation, the applicant demonstrates that *Brassica napus* and *Brassica rapa*, from which Isolexx is derived are not associated with mustard in the EU Commission common catalogue, an EFSA opinion on food allergens nor in the Codex standard for named vegetable oils limits. In addition, the applicant references scientific literature that supports the view that there is limited, if

any cross-sensitivity between mustard and non-mustard *Brassica* family members. In light of this, the FSAI is of the opinion that mustard allergen labelling is not required.