FOOD STANDARDS
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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Risk assessment (at Approval) – Proposal P1026

Lupin as an Allergen

Executive summary

Lupin is a legume increasingly used in food around the world. The nutritional properties of lupin are being recognised and technological applications are extending the use of lupin in food. In Australia, various locally made and imported lupin-containing food products are available to consumers. Lupin bran and flour are used in staple foods, such as bread and pasta, and confectionery. Also, a wide range of lupin-derived ingredients are in various stages of commercial development. While many existing uses of lupin in food are declared in ingredient labelling on food labels, some current and future applications may not be captured by these labelling requirements, potentially meaning the presence of lupin ingredients in food is not always declared to consumers.
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In Europe, lupin allergy is well documented in the medical literature including case reports of severe allergic reactions to lupin in a range of food products, and clinical studies using double blind placebo-controlled food challenges (DBPCFC). Lupin has been recognised as a significant allergen in the European Union food regulations since 2007, and in other jurisdictions.

Lupin allergy was first reported in the medical literature in Australia in 2004. Severe allergic reactions, including anaphylaxis, to lupin and lupin-containing food products have been reported from South Australia, Western Australia and the Australian Capital Territory. The Lupin Anaphylaxis Register currently has 14 well-documented cases. The prevalence of lupin allergy in the general population in Australia and New Zealand is unknown. However, the estimated rate of lupin sensitisation among patients who respond to a range of foods by the skin prick test is reported to be 4% in the <1 year age group and up to 25% in the >15 year age group. Lupin challenge studies in patients with known peanut allergy show that 25% of lupin-sensitised children and 41% of adults reacted to lupin. These results suggest under-reporting of lupin allergy in Australia possibly due to limited testing and dietary exposure. This information has been used to evaluate the significance of lupin against international criteria to identify new allergens (WHO, 2000).The allergenicity potential of lupin and derived substances is not destroyed by common food processing methods. Allergic reactions to lupin, based on EU and Australian evidence, fulfil international criteria for it to be classed an allergen of public health significance. Although the presence of lupin in food is currently limited, it is likely to increase and the potential for lupin ingredients to be present in food without being declared to consumers is high. The outcome confirms that, in Australia, lupin is a significant new allergen that presents a risk to allergic consumers.

Summary evaluation of the public health significance of lupin as a new food allergen	

1. Clinical evidence on lupin allergy	Lupin allergy in Australia:
	
	 a. Case reports:
	 • 3 cases (published Smith et al, 2004)
	 • Symptoms of severe reactions after consumption of bread roll containing lupin,
	 • Skin prick test (SPT) results: 3 positive (≥3mm),
	 • Lupin specific serum IgE results (Unicap1 positive, 2 not done)
	 • Region: South Australia
	
	 b. Lupin Anaphylaxis Register:
	 • 14 cases (unpublished, W. Smith)
	 • SPT results (13 of 14 reported)
	 • Lupin specific serum IgE results (RAST 8 of 14 reported)
	 • Region: 11 South Australia + 3 Australian Capital Territory
	 c. Clinical studies:
	
	 • 10 patients recruited from clinic or lupin processing factory, (published-Goggin et al., 2008)
	   ➤ History of reacting to lupin in food
	   ➤ Symptoms reported
	   ➤ SPT and/or serum IgE test results
	   ➤ Immunoblots with lupin flour
	   ➤ Region: Western Australia
	
	 • Lupin sensitization in a high risk population (unpublished-Loblay et al., 2009)
	   ➤ SPT: 14.5% sensitised to lupin
	
	 • Lupin food challenge studies in patients with peanut allergy (unpublished-Loblay et al., 2009)
	   ➤ 25% of lupin-sensitised children and 41% of adults reacted to lupin
	
	 • Regions: New South Wales and Western Australia

2. Information on current and potential use of lupin in food	• Lupin is listed as an ingredient in products currently available to consumers in Australia, including staple foods such as pasta, and some imported products. • Lupin is also used in Australia in unpackaged bakery products such as bread and muffins. • There is a wide range of ingredients in various stages of development through lupin R & D programs in WA.

Summary evaluation of the public health significance of lupin as a new food allergen		
3. Assessment against criteria	Criteria: WHO (2000) The existence of a credible cause and effect relationship based upon positive reaction to a DBPCFC, [cause and effect]	yes
	or unequivocal reports of reactions with typical features of allergic or intolerance reactions [immune-mediated reaction]	yes
	Reports of severe systemic reactions after exposure to the foodstuff [severity/ symptoms]	yes
	Data on the prevalence of the food allergies in children and adults, supported by appropriate clinical studies (i.e. DBPCFC) in the general population of several countries. However, the Panel noted that such information is available only for infants from certain countries and for certain foodstuffs. The Panel therefore agreed that any available data, such as the comparative prevalence of a specific food allergy in groups of patients in several countries, could be used as an alternative, preferably backed up by the results of DBPCFC [prevalence]	Prevalence data limited sensitization and allergy among clinic patients (1.a. above)
	Revised criteria Require confirmation that the food causes IgE-mediated reactions based on DBPCFC and serological evidence	yes
	Potency of the allergen: less than peanut, [VITAL action level 4.0 mg lupin protein vs 0.2 mg peanut protein]	yes
	The revised criteria also take into account additional factors including: ➢ use in food: some information is available	yes
	➢ impact of processing on potency of allergen: allergenicity not reduced by common processing, e.g. heat	yes
	➢ any cross-reactivity with known allergens: possible cross-reactivity with peanut (in Europe), cross-reactions with peanut not confirmed in Australia	not confirmed

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1   Introduction

The allergen review recognised that, with a continuously evolving food supply, new food allergens may emerge that would need to be considered for mandatory declaration. The review identified lupin as an emerging allergen in Australia and recommended that FSANZ considers the available evidence to determine whether lupin should be added to the list of substances subject to mandatory declaration requirements (section 1.2.3—4).

The purpose of this document is to evaluate the public health significance of lupin as a new food allergen in Australia and New Zealand against international criteria for new allergens. The evaluation is based on a review of the clinical evidence of lupin allergy in Australia and New Zealand, with reference to European data. The evaluation also takes into account information on current and potential use of lupin in food in Australia and New Zealand.

2   Lupin use in food

Lupin is a member of the legume family like peanut, soy, pea, bean and lentil. There are 12 lupin species within the *Lupinus* genus, all of which are native to Europe and the Mediterranean region. The 3 main species used in food are *Lupinus albus* (white lupin), *L. luteus* (yellow lupin) and *L. angustifolius* (blue or Australian sweet lupin), with the latter being a major crop in Western Australia (Sipsas, 2008). Lupin is a good source of nutrients being rich in proteins, lipids, dietary fibre, minerals and vitamins (Kohajdová et al, 2011). Since the late 1990s, lupin flour has been used to supplement staple foods such as bread and pasta in Europe. Lupin products have also entered the food supply in Australia (Woo, 2008). Current and potential food applications of lupin include baked foods such as breads, cakes and muffins; vegetarian products; whipped products, fillings and glazes; ice cream, desserts, mayonnaise and dressings, high protein energy drinks, and lupin protein concentrates and isolates for use as binding and emulsifying agents (Sipsas, 2008).

3   Outline of clinical data on lupin allergy in Australia

3.1   Lupin anaphylaxis cases

In 2004, three case reports of anaphylaxis to lupin in Adelaide (South Australia) were published (Smith et al, 2004)). Two of the patients suffered severe allergic reactions requiring hospitalisation after consuming bread rolls containing lupin bran. Skin prick testing performed on the patients, using saline extracts of lupin bran, were strongly positive. The third patient developed severe respiratory symptoms after consuming commercially prepared whole lupin, and less severe symptoms after consuming home prepared boiled and salted lupin, imported lupin-containing biscuits and a bread roll (the presence of lupin in the bread was suspected but not confirmed). None of the patients was allergic to peanut at that time. Once the allergy was determined, the patients were advised to avoid lupin and lupin products.

Since these initial reports, a register of lupin-induced anaphylaxis has been maintained by Dr William Smith at the Royal Adelaide Hospital (Dr W Smith in Loblay et al, 2009-unpublished data). The register collates anonymous data on patient location (State/ Territory), year of birth, gender, year of reaction, grade of reaction, food trigger, peanut allergy history, results of lupin skin prick test (SPT) and blood test.

There are 14 cases recorded in the Lupin Anaphylaxis Register: 10 cases in South Australia and four cases in the ACT. The following is a summary of data from the Australian Lupin Anaphylaxis Register:

Total as of December 2014	14 cases (including 3 cases in Smith et al, 2004)
Severity of reactions reported in register	• Anaphylaxis-moderate (9 cases) • Anaphylaxis-severe (4 cases) • Acute cutaneous reaction (1 case)
Demographics	• 13 adults (11 Female, 10 SA, 3 ACT) • one child (Female, ACT)

In 6 of the 14 cases recorded in the register, the food that triggered the allergic reaction was lupin seeds home-cooked or purchased pre-cooked and preserved in brine. All other cases were triggered by processed food of multiple ingredients, including specialty bread (5 cases), imported chocolate (3 cases), and one case where the food triggering the reaction was unknown but pasta/ pastry was suspected. However, in all cases, the route of sensitisation to lupin was unknown, and only one patient was SPT positive to peanut with a history of peanut allergy. All lupin-allergic patients were advised to avoid lupin and lupin-containing food products. No additional reports of cases were made after two calls appeared in a professional newsletter. The lupin anaphylaxis register has not been actively maintained since this time.

There is no information on the incidence and severity of lupin allergy in other parts of Australia, although lupin anaphylaxis cases are reported to have been recognised in Western Australia in the 1990s (W Smith, in Loblay et al, 2009-unpublished data). Testing for lupin allergy is not common practice in most allergy clinics around Australia. Allergy clinicians have suggested that due to lack of routine testing, lupin allergy may be under-reported (W Smith and R Loblay, personal communication).

3.2   Lupin sensitisation

Sensitisation is the initiation of the allergic process. It occurs when an allergen stimulates the immune system to produce specific IgE antibodies. Sensitisation, commonly measured using the SPT, is regarded as a risk marker for developing allergy symptoms. However, sensitisation may or may not lead to clinical allergy. Confirmation of clinical relevance is based either on convincing history of allergic reactions to the specific food, or positive reactions in oral food challenges. Some people develop IgE antibodies but do not react to ingested lupin. Lupin sensitisation can occur via inhalation of lupin flour or via ingestion of lupin and lupin products, or possibly (unproven) through application of lupin-containing products (eg cosmetics) to the skin.

In Australia, a pilot investigation into lupin allergy was commenced in 2007. Clinical studies were conducted at the Royal Prince Alfred Hospital (RPAH) in Sydney, NS W, and the Princess Margaret Hospital for Children in Perth, WA. The aim of these studies was to gather information on the prevalence of lupin sensitisation in a high-risk clinic population, and determine the clinical reactivity, particularly in peanut allergic individuals. A summary report on these studies was provided to FSANZ (Loblay et al, 2009-unpublished data). Results from the report are outlined below:

3.2.1   RPAH allergy clinic population (Sydney group)

•   Data on lupin sensitisation were collected over 3 years and analysed according to age groups: <1, 1, 2−5, 6−15 and >15 years.

- In a total of 6006 clinic patients tested for lupin sensitisation, 6.5% were SPT positive (SPT ≥3x3 mm).

- Of 2924 patients who were SPT positive to any food, 14.5% were sensitised to lupin. The rate of lupin sensitisation increased with age from 4% in the <1 year age group to 25% in the >15 year age group.

- Allergy clinic patients (number unspecified) were tested for sensitisation to legumes (lupin, peanuts, soy and pea) and to tree nuts (cashew, almond and hazelnut). The rate of lupin sensitisation was found to be comparable to that of soy across all age groups, peaking at 13% in the 6−15 years age group.

- The study also investigated lupin co-sensitisation in patients grouped according to their sensitisation to peanut, cashew, almond, hazelnut as well as sesame, wheat, soy, egg and milk. The results show that, generally, lupin co-sensitisation increased with age in all groups.

- Lupin co-sensitisation was most common in the wheat, almond and soy sensitised groups peaking at 53.8%, 43.3% and 37%, respectively, in the 6−15 year group.

- In the peanut, cashew, hazelnut, sesame and egg sensitised groups, the percentage of lupin co-sensitised patients was comparable across the age groups.

- Focusing on the group of patients who were sensitised to peanut, the study compared co-sensitisation with the following allergenic foods: tree nuts (cashew, almond, and hazelnut), legumes (lupin and soy) as well as sesame, wheat, soy, egg and milk. The results show that, among the peanut sensitised, co-sensitisation to lupin was at 13% in the 1 year age group increasing to 37% in the over 15 year old group, and a similar pattern of soy co-sensitisation was reported.

3.2.2   Lupin sensitisation /allergy reported in other regions of Australia

Perth, Western Australia data

Allergic reactions to lupin in food have been reported in Western Australia

Goggin et al (2008) reported data on twelve subjects (confirmed to be in WA, Dr Martin Stuckey, personal communication). The subjects were mostly recruited from workplaces involved in lupin research or in processing lupin flour, or had presented to medical clinics with anaphylaxis. Ten of the twelve subjects were reported to be allergic to ingested lupin products, including two who were also allergic to inhaled lupin products. Of the ten subjects who were allergic to ingested lupin, nine had positive SPT results to lupin seed extract (wheal diameter of ≥ 3 mm). The allergy status of one subject, who was not skin tested, was confirmed based on history of multiple reactions to foods where lupin was the common ingredient, as well as a high level of lupin-specific IgE. Sera from subjects, for whom data were available, contained high or very high levels of lupin-specific IgE. Western blot results using *L. angustifolius* flour probed with 8 sera showed IgE reactive bands, mainly in the range of 49−90 kDa. The study suggests that conglutin-β is a major allergen for *L. angustifolius*, because IgE from all sera, where IgE was detectable, bound the purified protein.

Five subjects had lupin-specific IgE, but further detail was unavailable to FSANZ at the time of preparing this report (Dr Martin Stuckey, personal communication).

ACT clinic – skin testing

An allergy clinic in the ACT shows that of 65 peanut-allergic patients skin-tested for lupin sensitisation, 7 patients were lupin positive (wheal size ≥ 3 mm), but only 2 patients were convincingly positive (≥ 8 mm). None of the patients were known to be lupin allergic, and oral food challenges were not conducted (Dr Ray Mullins, personal communication).

Melbourne clinic – skin testing

Skin testing with lupin in a paediatric allergy clinic in Melbourne was conducted during 2011. As of August 2012, no positive results were reported (Prof Katie Allen, personal communication).

Adelaide clinic – skin testing

Data on skin testing for lupin sensitisation in allergy clinic population over a seven-year period from 2005−2012 was provided to FSANZ (Dr Frank Kette and Dr William Smith, personal communication). Overall, the data show a steady rise in lupin sensitisation over this period, from <2% in 2005−2006 to 6% in 2011−2012 (data for 2009-2010 <2%, possibly due to inactive lupin extract). Data also show that in 2011−2012, the lupin sensitisation rate of 6% is about half the rate of peanut sensitisation in the same clinic population.

3.3 Lupin challenge studies in peanut sensitised/ peanut allergic patients

Published reports of lupin allergy in Europe suggested that peanut allergic individuals are at a high risk of reacting to lupin due to cross-reactivity (discussed further in Section 4).

To investigate this further, Australian researchers sought to determine the prevalence of lupin allergy among peanut sensitised and peanut allergic patients in Sydney and Perth (Loblay et al, 2009-unpublished data). The study included 134 SPT positive patients: 112 children up to 15 years of age (Sydney 80 and Perth 32), and 22 adults aged 16-60 (Sydney 21, Perth 1). Of the Sydney participants, 70% were peanut allergic and 30% were peanut sensitised. All Perth participants were peanut allergic. Lupin SPT positive status was confirmed on the day of the challenge.

An initial 'lip challenge' was conducted and no further challenge was given in the case of reactivity. If the initial challenge was negative, subjects proceeded to the oral challenge which continued until there was a positive reaction. Oral challenges were conducted at 15 minute intervals, using incremental doses of lupin from 0.01 g to 6.4 g (cumulative dose of 12.76 g).

In this study, a positive challenge was defined as any objective evidence of clinical reactivity during the challenge including angioedema, urticaria, wheeze, stridor, hoarse voice, vomiting or cardiovascular disturbance.

The following is a summary of the oral challenge results:

- Of the 112 children challenged with lupin:

    - 28 children (25%) had some form of clinical reaction, including four children who required adrenaline due to evidence of cardiovascular or respiratory compromise.
    - the challenge was fully tolerated by 84 children (75%).

- Of the 22 adults challenged with lupin:

  – 9 patients (40.9%) had a clinical reaction that was judged to be significant (challenge was terminated to prevent progression).
  – 13 patients (59.1%) tolerated the challenge without reaction.

- The challenge data for 20 children and 10 adults from the Sydney group show that:

  – 12 patients (7 children and 5 adults) reacted to a cumulative dose of <1 g, including 3 patients (2 children and 1 adult) reacted to the first dose of 0.01g.
  – 12 patients (9 children and 3 adults) reacted to cumulative doses between 1.56 g and 6.36 g.
  – 4 patients (3 children and 1 adult) reacted to a cumulative dose of12.76 g.

3.4 Conclusions from the clinical investigation of lupin allergy in Australia

In the report provided to FSANZ (Loblay et al, 2009-unpublished data), the authors concluded that their studies have demonstrated the following:

- Lupin sensitisation is common in atopic children and adults presenting to specialized clinical allergy services, and prevalence increases with age.

- Co-sensitisation between lupin and other food allergens is common, and is not confined to peanut. In all age groups, the rate of lupin co-sensitisation in peanut sensitised patients is comparable to that in patients sensitised to cashews, hazelnuts, sesame and egg. Up to the age of 15, the lupin co-sensitisation rate is comparable in milk-sensitised patients and significantly higher in those sensitised to almond, wheat and soy.

- Of those sensitised, 25% of children and 40% of adults developed clinical reactions to challenge. Four required treatment with adrenaline. In the Sydney group, 22% developed delayed reactions, mostly with gastrointestinal symptoms.

- Apart from those with lip contact reactivity, threshold doses for challenge reactions are relatively high.

- There is good correlation between lupin SPT size, quantitative RAST to lupin and the probability of clinical reaction to challenge.

- Sensitisation and clinical allergy to lupin (including anaphylaxis) can occur in the absence of peanut sensitisation.

- Clinical reactions to ingestion of trace amounts of lupin have not been documented.

- Ig from individuals sensitised to lupin alone predominantly bound to conglutin β while that from individuals co-sensitised to peanut and lupin did not.

- Characterisation of the proteins bound by Ig from peanut/lupin sera suggested that these individuals reacted 25kDa proteins that were identified as either conglutin-α or γ.

Among the common food allergens, sensitisation and clinical allergy to lupin in children appears to be most comparable in frequency and severity to soy. Although lupin allergy is commonly seen in association with peanut allergy, it is equally common in children sensitised to tree nuts and to egg, and may also occur as an isolated phenomenon without peanut sensitisation. Severe reactions have been documented, particularly in adults sensitised to lupin alone.

3.5   Lupin allergy in other parts of Australia and New Zealand

FSANZ is not aware of any published or unpublished reports of allergic reactions to lupin in food, or clinical studies on the prevalence of lupin sensitisation or lupin allergy, in other locations in Australia or in New Zealand.

4   Lupin allergy and the role of legume cross-reactivity

The inclusion of lupin in food was authorised in the UK and France in 1996-97. As lupin use in food increased in Europe, allergic reactions to various food products containing lupin were increasingly reported. By 2002, lupin has become the fourth most frequent cause of food-associated anaphylaxis reported to the French Allergy Vigilance Network (Moneret-Vautrin et al, 2004). In 2005 the European Food Safety Authority (EFSA) published an opinion on lupin allergy which highlighted the increasing consumption of lupin, the reported incidence and severity of allergic reactions to lupin, and the risk of clinically relevant cross reactivity in peanut allergic individuals (EFSA, 2005). In 2007 lupin was added to the list of food allergens in the European Union (EU) according to the Commission Directive 2006/142/EC amending Annex IIIA of Directive 2000/13/EC.

In Europe, lupin allergy is now well documented in the medical literature including case reports of severe allergic reactions to lupin in a range of food products, and clinical studies using double blind placebo controlled challenges (DBPCFC). Lupin allergy may result from primary sensitisation to lupin, or from cross-reactivity in individuals allergic to other legumes, such as peanut and soy. Sensitisation to lupin is frequently asymptomatic; lupin allergy can manifest for the first time in adults in clinical reactivity ranging from severe anaphylaxis to urticaria and vomiting (Bansal et al, 2014). However, early cases of lupin allergy were mainly reported in patients known to be allergic to other legumes, particularly peanut. These results drew attention to the potential risk of allergic reactions to lupin among peanut allergic individuals.

A significant body of evidence now exists on lupin allergy in Europe. Since 1999, numerous reports of allergic reactions to lupin in a wide range of food products have been published from many European countries including France, the UK, Spain, Italy, the Netherlands, Germany, Denmark and Norway. A review of the literature, published in 2010, identified at least 151 cases of lupin allergy worldwide (Jappe and Viet's, 2010).

The first report of lupin allergy was in 1994 and involved a 5 year old girl with a known peanut allergy who developed urticaria and angioedema after eating pasta fortified with lupin flour (Hefle et al, 1994). The study also reported positive skin and serum tests to lupin in five out of seven peanut allergic adult patients, but the clinical relevance of lupin sensitisation in these patients was not investigated. The patients who had a positive skin test to lupin also reported a history or adverse reactions to green pea. The lupin proteins recognised by serum IgE from these patients were 21 kDa and 35–55 kDa.

In France, serological and clinical cross reactivity to lupin were investigated in 24 peanut allergic children (Moneret-Vautrin et al, 1999). Eleven patients were sensitised to lupin (44%). Eight patients were tested for allergy to lupin using the labial challenge and DBPCFC.

Seven patients had positive reactions, including two to the labial test. Immunoblot analysis of lupin proteins using sera from five patients showed a distinct IgE band at 43-kDa. The study also showed immunoblot inhibition caused by peanut extract. The authors suggested that peanut allergic individuals may be at increased risk of cross-reacting to lupin. Analysis of the 107 cases of severe food anaphylaxis registered in 2002 by the French Allergy Vigilance Network, identified lupin as the fourth most common cause of food anaphylaxis (Moneret-Vautrin et al, 2004).

These early results raised concerns of potential clinically relevant cross-reactivity between lupin and peanut, and prompted further investigations on the prevalence of lupin allergy in peanut allergic patients.

The following is an outline of some of the clinical studies reported from European countries.

A UK study investigated the prevalence of lupin sensitisation and lupin allergy in children and teenagers allergic to peanut (Shaw et al, 2008). The results indicate that 16 of 47 peanut allergic patients were sensitised to lupin, similar to the French study. However, the prevalence of lupin allergy was low compared to the French study, with only 2 of 9 patients reported to react in the DBPCFC with lupin.

In Denmark, a study of 39 peanut-sensitised adults found 20 of those patients were also sensitised to three other commonly consumed legumes (lupin, soy and pea), 37 were sensitised to at least one of these three legumes. Only two patients were not sensitised to any of these 3 legumes. The study also reported sensitisation was at 82% to lupin, 87% to soy and 55% to pea (Peeters et al, 2009). Based on patient history, the study reported allergy to peanut at 74%, soy at 33% and pea at 29%. Allergy to lupin was confirmed by DBPCFC in 35% of cases; but no predictive factors for lupin allergy could be identified. The results show that, in peanut-sensitised patients, the pattern and frequency of sensitisation and allergy for lupin and for soy were similar. Whether these results reflect co-sensitisation or cross-reactivity is unknown. Notably, none of the 8 patients who had a positive reaction to lupin were aware of their lupin allergy or that lupin is used in some foods (Peeters et al, 2009).

A Norwegian study investigated lupin allergy in a group of 35 children referred to a clinic over a 3-month period for known or suspected food allergy, including one child for suspected lupin allergy (Lindvik et al, 2007). Fifteen of 35 children (43%) had positive SPT to lupin. All children sensitised to lupin were also sensitised to one or more of the three other legumes tested for, i.e. peanut, pea and soy. Of the 15 children SPT positive to lupin, 9 were SPT positive to peanut, 5 to pea and 8 to soy. Twenty-eight children had peanut specific IgE, and of these, 17 children had IgE specific to lupin, 15 to pea and 16 to soy. Ten of the 15 children with positive SPT to lupin underwent oral food challenges and one child experienced an allergic reaction. The child had IgE specific to lupin and to peanut. The study also showed that sensitisation to pea, or soy, occurred more often than peanut sensitisation in the children sensitised to lupin. However, the Norwegian Food Allergy Register received a number of reports of peanut allergic patients experiencing serious reactions to lupin in food during a ten-year survey (Namork et al, 2011).

In Spain, a case of lupin allergy was reported in a patient allergic to peanut and lentil prompted an investigation of reactivity to lupin in lentil allergic patients (Cabanillas et al, 2010). Five consecutive lentil allergic patients were recruited, all of whom stated they have not knowingly ingested lupin previously. The results showed that 4 patients were sensitised to lupin and 3 patients were allergic to lupin. Immunoblot inhibition experiments using sera from 2 patients suggest that lupin was the primary sensitiser in one patient with onset of lentil allergy later in life. In contrast, lentil appears to be the primary sensitiser in another patient who developed lupin allergy later in life.

The results indicate that in addition to cross-reactivity, primary sensitisation due to unrecognised dietary and/ or environmental exposure to lupin account for lupin allergy.

In Finland, a study investigated sensitisation to lupin flour in 1,522 patients with suspected food allergy from November 1, 2005, through December 31, 2007 (Hieta et al, 2009). The results show 25 of 1,522 patients (1.6%) had positive SPT reactions, and probable lupin allergy was diagnosed in 7 of 25 patients, in whom the clinical symptoms varied from anaphylaxis and respiratory symptoms to contact urticaria and itchy mouth. Cross-reactions or concurrent reactions to other legumes were seen in 18 of 25 patients.

In Germany, SPTs were performed with lupin, pea, peanut and soybean in 81 atopic and 102 non-atopic adults (Bahr et al, 2014). Discounting invalid responses in 20 subjects, the results for 163 subjects were analysed. Of these, 18 had a positive reaction to at least one legume tested. Overall, six subjects (4%) were sensitised to lupin, 12 (7%) to pea, five (3%) to peanut and eight (5%) to soybean. Of the six subjects sensitised to lupin, three (50%) were also sensitised to pea, three (50%) to peanut, and five (83%) to soybean. Lupin sensitisation was demonstrated in only 2% of the non-atopic subjects. Subjects with existing sensitisation or allergy to other legumes were considered to be at higher risk for a sensitisation or allergy to lupin due to cross-reactivity.

In addition to potential cross-reactivity, several studies have reported lupin allergy as a separate entity, without evidence of clinical or serological cross-reactivity to other legumes (Smith et al, 2004; Jappe and Vieths, 2010; Peeters et al, 2007). A review of 102 cases of lupin allergy reported in the literature, found that pre-existing peanut allergy was only documented in 48 cases (Jappe and Vieths, 2010). Therefore, lupin is an important primary food allergen as well as a potentially cross-reactive allergen. Primary allergy to lupin is particularly relevant to Australia, where 13 of 14 lupin anaphylaxis cases recorded in the register were not peanut allergic (W Smith in Loblay et al, 2009).

5   Lupin proteins and identified allergens

Compositional analysis of *L. angustifolius* shows a high protein content of up to 40% (of the total weight of the kernel). Most of the protein consists of globulin-type storage proteins called conglutins. Four conglutin fractions have been identified as: conglutin-α (legumin-like protein), conglutin-β (vicilin-like protein), conglutin-δ, and conglutin-γ. The conglutins account for up to 85% of the total protein and the remaining 15% are albumins (Guillamón et al, 2010). Conglutin-β and conglutin-α are the two main conglutin proteins. Conglutin-β comprises up to 12 major subunits and a number of minor subunits ranging from 15-65 kDa. Conglutin-α contains four types of subunits each comprising a heavy (31-46 kDa) and a light polypeptide chain of 19kDa, linked by two disulfide bridges (Lqari et al, 2004).

A number of studies have shown the presence of several important IgE reactive proteins in lupin. An Australian study identified β-conglutin as the major allergen in *L. angustifolius* (Goggin et al, 2008)*.* Sera from 12 patients with respiratory allergy and food allergy to lupin were included in mass spectrometric analysis of IgE-reactive protein spots on two-dimensional gels, and IgE specific reactivity of purified conglutin-β. The results indicate that all sera in which IgE could be detected (8 of the 12 patients), recognised the purified conglutin-β protein. This allergen has been officially designated Lup an 1 by the International Union of Immunological Societies (IUIS). Conglutin-β was also confirmed as an allergen in *L. albus*, and possibly *L. luteus* (Goggin et al, 2008)*.*

A European study identified two major allergens in *L. albus*, as Lup-1 and Lup-2, corresponding to conglutin-β and conglutin-α, respectively (Guillamón et al, 2010). The study also showed that Lup-1 and Lup an 1 from *L. angustifolius* were highly homologous.

Sequence homologies between conglutin-α and the peanut allergen Ara h 3; and between conglutin-β and Ara h1 have also been reported, which may explain the cross-reactivity between lupin and peanut (Guillamón et al, 2010; Sirtori et al, 2011).

An Italian study characterised the lupin protein sensitisation pattern in a group of 12 children allergic to peanut, to identify specific lupin proteins involved in cross-reactivity with peanut allergens (Ballabio et al 2013). Reactivity was measured by *in vitro* immunoblotting and *in vivo* fresh food skin prick test (FFSPT). The results showed conglutin-β was recognised by cutaneous IgE antibodies from seven out of the 12 peanut-allergic subjects in FFSPT, and by serum IgEs from five of the subjects. Four and eight subjects respectively tested positive to conglutin-γ in the SPT and immunoblot. In this group of children, conglutin-β was found to be the major lupin allergen involved in cross-reactivity with peanut allergenicity.

Based on serological reactivity, data from European studies suggest a predominant role of conglutin-α in the allergenicity of both *L. albus* and *L. angustifolius*; while in the Australian study, conglutin-β was identified as the predominant allergen (Guillamón et al, 2010; Goggin et al, 2008).

6 Effect of food processing on lupin allergenicity

Like other legume allergens, lupin allergens are relatively resistant to thermal, chemical and proteolytic degradation. Using IgE binding assays, researchers found the allergenicity of lupin was retained after extrusion cooking, boiling, autoclaving and microwave heating. Significant reduction in IgE binding was observed only after autoclaving at 138°C for 20 minutes (Alvarez-Alvarez et al, 2005). Therefore, lupin allergenicity would not be reduced in the majority of food products made using common food processing technologies.

7 Detection and quantification of lupin protein in food

The enzyme-linked immunosorbent assay (ELISA) is the most widely used for detection and identification of food allergens. ELISA kits for the detection of lupin protein in food have been commercialised by different suppliers. The ELISA SYSTEMS kit was developed specifically to detect European as well as Australian lupin species, making it suitable for locally produced as well as imported food products. The performance of ELISA SYSTEMS assay was evaluated, and reported to compare favourably in a range of processed food products (Treolar et al, 2009).

7.1 Establishing a Reference Dose

As part of the VITAL (Voluntary Incidental Trace Allergen Labeling) program of The Allergen Bureau of Australia and New Zealand, an expert panel was convened in 2011 to establish appropriate Reference Doses (or population thresholds) for allergenic food residues. Using published data on individual NOAELs and LOAELs for 11 allergenic foods, Reference Doses were estimated using interval censoring survival techniques (a statistical dose-distribution model) to which a log-normal, log-logistic or Weibull curve had been fitted (Taylor et al 2014).

Published food challenge studies (i.e. DBPCFC) with lupin yielded useable data from only 15 adults and nine children. For these food-challenge studies the administered protein dose was calculated by assuming the protein content of lupin flour was 36.2% or 40% if it involved yellow lupin flour. As lupin was one of the foods for which fewer data points were available, the 95% lower confidence interval of the ED_{05} (eliciting dose) was estimated using only two different dose-distribution models (log-normal, log-logistic).

From the analyses, the VITAL Reference Dose for lupin was estimated to be 4 mg protein; this compares with 0.2 mg for peanut and 1 mg for soy flour.

Lupin allergy is less common than allergy to other legumes. The small number of data points used in the analyses will have a significant effect on the reference dose estimate. However, individuals with lower thresholds for clinical reactivity and therefore at higher risk would almost certainly be under medical supervision and following appropriate dietary advice.

8 Lupin cross-contamination

There is limited information on lupin cross contamination in the commercial food supply in Australia. A preliminary investigation to determine the extent of lupin cross-contamination in bread was conducted by analysts at FACTA Pty Ltd. Thirty samples of bread, commercially available from retailers in southwest Brisbane, were tested for lupin using the ELISA method. Lupin was detected in 11 out of 30 samples. The detected levels were as follows:<2.5 ppm in 10 samples, and at >5.0 ppm in one sample. The source of lupin cross-contamination in bread was not determined (R Sherlock, personal communication).

At the food manufacturing level, lupin cross-contamination needs to be managed in the same way as other food allergens to minimise inadvertent exposure by allergic consumers. The Australian Allergen Bureau has developed guidance to food manufacturers to inform allergen control practices and to minimise the use of precautionary statements.

9 Criteria for identifying new food allergens of public health significance

From early on, the need for a scientific and transparent approach to identifying new food allergens of public health importance, has been recognised. In 1995, an expert consultation convened by the Food and Agriculture Organization identified eight foods as the most common causes of food allergy. (FAO,1995). The main criterion for inclusion was the frequency of reported reactions. In 1999, the Codex Alimentarius Commission adopted the list, known as the big 8, i.e. gluten-containing cereals, crustacea, fish, egg, milk, peanut, soy, and tree nuts. The list also included sulphite preservatives when added at ≥10 mg/kg.

The list was adopted into *Australia New Zealand Food Standards Code* based on the advice of a panel of allergy experts convened by FSANZ's predecessor (the Australia New Zealand Food Authority). The panel also recommended the inclusion of sesame seeds due to increasing clinical reports of severe reactions to sesame products, including anaphylaxis, among infants. The panel considered the prevalence and severity of allergic reactions to be the main criteria for identifying allergenic foods for regulatory purposes. However, the expert panel noted that data on prevalence are often limited and defined 'severe' reactions as those which lead to significant morbidity and mortality.

In 1998, ILSI Europe reviewed the Codex list and proposed two key criteria, i.e. allergenicity as confirmed by properly conducted DBPCFC studies, and severity of the reactions. Thresholds for eliciting doses and processing factors were recognised as important, but were not included due to lack of data at the time (Bousquet et al., 1998).

In 1999, criteria for new food allergens were proposed by an expert panel convened by the WHO (WHO, 2000). The criteria are:

1.   The existence of a credible cause and effect relationship based upon positive reaction to a DBPCFC, or unequivocal reports of reactions with typical features of allergic or intolerance reactions.

2.   Reports of severe systemic reactions after exposure to the foodstuff, the reactions including atopic dermatitis, urticaria, angio-oedema, laryngeal oedema, asthma, rhinitis, abdominal pain, diarrhoea, vomiting, anaphylactic shock and chronic severe malabsorption syndrome.

3.   Data on the prevalence of the food allergies in children and adults, supported by appropriate clinical studies (i.e. DBPCFC) in the general population of several countries. However, the Panel noted that such information is available only for infants from certain countries and for certain foodstuffs. The Panel therefore agreed that any available data, such as the comparative prevalence of a specific food allergy in groups of patients in several countries, could be used as an alternative, preferably backed up by the results of DBPCFC.

Although these criteria were developed for the purposes of an international body (i.e. the Codex Alimentarius), they are suitable for application by national regulators, taking into account specific information relevant to their jurisdiction. This is achieved by considering information on the prevalence of the allergy in the local population, and use of food and dietary exposure of the relevant population.

Over the years, the criteria have been further discussed and elaborated focusing on IgE-mediated reactions due to their potential to be severe and life-threatening (Bjőrkstén et al., 2008; van Bilsen et al., 2011).The revised criteria support the identification of allergenic foods of public health significance, by including confirmation that the food causes IgE-mediated reactions based on DBPCFC and serological evidence, and potency of the allergen. The revised criteria also take into account additional factors including use in food, impact of processing on potency of allergen, and any cross-reactivity with known allergens.

10   Evaluating lupin as a new allergen of public health significance in Australia

Over the past decade, a significant body of evidence has been published mostly based on European data. There is evidence for cause and effect and IgE-mediated mechanism based on DBPCFC and serological studies confirming that lupin can cause IgE-mediated reactions. Reactions to lupin reported in the literature include severe and anaphylactic reactions. In relation to potency, the available information indicates that the allergenic lupin proteins are heat stable. Also, as for other known allergens, relatively small amounts of lupin can trigger allergic reactions. There is some information to provide an estimate of prevalence in some European countries, particularly where lupin is more commonly consumed.

In Australia, lupin continues to gain recognition for its nutritional and technological properties and various new products are entering the food supply (Sipsas, 2008). Lupin food products are increasingly available from commercial outlets in Australia, including specialty food shops as well as from major food retailers (Woo, 2008).

In the past decade, 14 cases of severe allergic reactions to lupin have been documented in the Australian Lupin Anaphylaxis Register established by Dr William Smith in Adelaide, South Australia. Only two geographical regions in Australia are represented in the register with 10 cases from South Australia, and four cases from the ACT. The majority of cases are adults (13 out of 14) with no evidence of allergy to peanut. In most cases the foods which caused the allergic reactions were reported as bread, confectionery and possibly pasta.

There is also information from published and unpublished clinical studies on lupin allergy in Australia. Ten patients from WA were diagnosed based on history of severe reactions to lupin ingestion, positive SPT and lupin-specific serum IgE. Immunoblots using patient sera identified conglutin-β, purified from *L. angustifolius*, as a major allergenic protein in this group of patients.

Preliminary data from a clinical study in Australia (outlined in section 3) indicates that the rate of lupin sensitisation among food allergy patients is high. Up to 25% of children and 41% of adults who are sensitised to lupin are potentially at risk of allergic reaction if they consume lupin products. However, the incidence of severe allergic reactions to lupin reported to the Anaphylaxis register is less than might be expected Australia-wide based on the preliminary results of the lupin challenges. Possible explanations include limited presence of lupin in foods currently available to consumers in Australia. Another explanation is under-reporting of allergic reactions to lupin due to lupin not being included in diagnostic testing and lack of awareness about the presence of lupin in food. The prevalence of lupin allergy in the general population in Australia is unknown at this time.

The route of sensitisation in Australia is unknown, and may be due to ingestion, environmental exposure to lupin pollen and lupin flour dust, or transcutaneous absorption. However, it is clear that the current level of exposure to lupin in Australia can lead to sensitisation and clinically relevant allergy to lupin-containing food products.

The clinical data from Australia on lupin allergy fulfils the international criteria for significant new allergens. This information should be taken into account together with the likely increase of lupin in the food supply.

11 Conclusions

Lupin is used in a wide range of food products in many countries particularly in Europe, and increasingly in Australia. In the international context, the public health significance of lupin as a food allergen in Europe is supported by a large body of evidence. In Australia, 14 severe allergic reactions to lupin have been documented in an anaphylaxis register. Data on lupin sensitisation suggest a steady increase over the past decade. In addition, unpublished data from a clinical study conducted in Sydney and Perth, suggest that a significant proportion of children and adults who are sensitised to lupin are potentially at risk of allergic reaction if they consume lupin products. This information leads to the conclusion that lupin is an emerging food allergen of public health significance in Australia, and together with the increasing use of lupin in food, would support the inclusion of lupin in the list of allergens subject to mandatory declaration in the Code.

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