Review of the food safety issues relating to the human consumption of lupins.

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1 EXECUTIVE SUMMARY

While lupin seeds have a long tradition of use for human consumption in some European and South American countries, lupins are more recently being manufactured in new and innovative ways. Lupin flour, for example, may be added to enhance the protein content and aesthetics of baked products. The increasingly widespread novel applications of lupins in the food supply are accompanied by an increase in the exposure of the general population to lupins.

Currently there is little medical literature pertaining to the risks of such exposure. However, a review of the literature does bear out two major concerns. The first centres upon the toxicity issue of the 'bitter' varieties imported from Southern Europe. One Australian woman presented to a Melbourne Emergency Room in 1995 after accidentally ingesting a toxic dose of lupin alkaloids. She was released after 24 hours in supportive observation. Fortunately, lupin alkaloid toxicity is rare in humans. The development of the 'sweet' varieties of lupins circumvents the toxicity issue but its widespread availability in the food supply brings the inauspicious issue of allergenicity.

A food allergen is a common protein capable of causing an allergic reaction in hypersensitive individuals. Although the allergen is usually intrinsically not harmful, the most severe forms of allergic reaction, anaphylaxis, can be fatal. Most people do not develop hypersensitivity reactions to allergens in foods, but an estimated 1% of the population develop food allergies.

Hypersensitivity is a pathophysiological reaction of the immune system, which occurs subsequent to an initial exposure to the allergen. For instance, it was demonstrated that it is possible for workers handling lupin flour to become sensitised to lupin via the respiratory system, which may subsequently lead to occupational asthma and food allergy.

The first evidence that lupins may be allergenic came from individuals with hypersensitivity to another legume, the peanut. The first case of lupin allergy was reported in 1994 in the US. It involved an adverse skin reaction of a 5-year old peanut-sensitive girl to a pasta product that had been fortified with lupin flour. The investigators then sampled seven peanut-sensitive adults and found that five also had positive skin prick tests to lupin-fortified pasta.

The allergens contained in lupin proteins are largely unknown. The molecular weight range of lupin proteins recognised by the IgE sera of peanut sensitive individuals was observed to be 21 kd and between 35-55 kd, but these allergens have not been fully characterised or named. They appear to be heat stable. Interestingly, a lupin flour immunoblot was totally inhibited by peanut at the 43 kd allergen, indicating structural similarities of these proteins. Although not a previously identified allergen in peanuts, the investigators claim it may be of clinical importance and may provide immunochemical evidence of a cross reactivity between lupin and peanut.

European studies later demonstrated results similar to the US study, showing high levels of positive skin prick tests, indicating IgE binding, in peanut-sensitive individuals. However, these findings must be tempered by the low reliability of skin prick tests to accurately predict clinically relevant hypersensitivity reactions.

This point was effectively demonstrated by a study that set out to determine the actual incidence of clinically important cross reactivity among legume hypersensitive individuals. Subjects were included if they had positive skin prick tests to one or more legumes (including peanut, soybean, pea, green bean and lima been). Out of 130 positive skin tests for legume hypersensitivity, only 43 double blind, placebo controlled oral challenge (DBPCFC) tests were positive. That is, only 33% of the positive skin tests were clinically relevant. Further, when the DBPCFC (the gold standard) was used to diagnose the incidence of cross-reactivity amongst the legume family, only 5% were found to be clinically significant.

However uncommon, if the clinical manifestation of such allergic reactions involves anaphylaxis then it has the potential to be fatal. With respect to lupins there have yet been no reported fatalities, but there are three cases of anaphylaxis to lupin proteins in the literature. Two were young children in a Parisian hospital for other reasons. Both children had a known peanut allergy and were given a chocolate milk drink for breakfast. Subsequently both children had experienced anaphylactic shock. Upon investigation, the product was found to contain lupin flour, although not labelled as such.

There are calls in Europe to label products containing lupins. Without adequate labelling, lupin proteins have the potential to become a ‘hidden’ or ‘masked’ allergen. Those particularly at risk appear to be: (1) peanut sensitive individuals, especially children and those with a history of adverse reactions to other legumes, such as green pea, and; (2) individuals working with lupin flour may be at risk of developing respiratory reactions or food allergy to lupin after being sensitised through occupational exposure.

In Europe there appears to be an increasing number of reported cases corresponding with the increasing exposure of sensitive individuals to lupin proteins, especially in the form of flour or dust. Although these initial reports concern only atopic or occupationally related individuals as those at risk, there are concerns that it is only a matter of time before isolated cases of primary sensitisation are reported. Lupin allergies were first reported in Australia at the time that this review was prepared. An article in the Medical Journal of Australia reported three cases of what appear to be severe allergic reactions to ingested lupins. This has highlighted the need for greater awareness of lupin allergies in Australia.

Too little is understood of the development of food allergy to novel proteins in the population. However, the incidence is increasing and children are especially vulnerable. This pattern, as observed with peanut and soy, is thought to reflect the early incorporation of novel proteins into the diets of children coupled with the nature of primary sensitisation to allergens to occur early in life.

Currently, lupin is not covered by mandatory labeling regulations such as are in place for peanut, soy and several other allergenic foods. Mandatory labeling may provide the best long term solution for the application of lupins or lupin products in food. Whilst the lowest dose required for an allergic reaction is unknown, it is recommended that in the absence of mandatory labeling, that products that may contain even traces of lupins, are labeled accordingly. It would also be vigilant to warn peanut sensitive consumers on the label that there may be a potential for problems. Implementation of these recommendations would: (1) provide an opportunity for post-market surveillance of these allergens; and (2) assist in the prevention of accidental exposure of an increasing number of sensitive individuals to these novel proteins. A further recommendation is for close relationships with organizations such as Anaphylaxis Australia to be maintained by the food industry to avoid accidental exposure of individuals at high risk.

2 INTRODUCTION

Lupins have been used as a food for humans and livestock for over 2000 years[1]. *Lupinus spp* contribute to soil nitrogen function and the seeds are a high quality source of protein, lipids, and dietary fiber. A factor limiting the consumption of lupin seeds has been the high alkaloid content (2-3%) [2]. The alkaloids confer a bitter taste and are toxic when ingested. Traditionally, a long “debittering” process was applied to remove the alkaloids. Recently low-alkaloid varieties (approximately 0.001%) have been selectively developed [3]. These varieties are non-toxic and are increasingly being incorporated into the world food supply in both traditional and novel ways [4].

Recently, however, there have been calls in Europe for caution in the bourgeoning incorporation of lupin seeds into the food supply [5]. An increasing number of reports suggest lupins may contain allergens [6]. A strong cross reactive potential between lupins and peanuts has been demonstrated in peanut-sensitive individuals [7]. Additionally, occupational exposure to lupin flour has lead to lupin hypersensitivity [8]. It may only be a matter of time before primary sensitisation to the lupin seed is reported [9]. The

literature pertaining to the adverse effects to humans of lupins including potential allergenicity is reviewed.

2.1 LUPINS IN HUMAN CONSUMPTION

2.1.1 TRADITIONAL USE

The people living around the Mediterranean and in the Andes highlands have a long traditions of lupin use for human consumption [10]. In the Mediterranean tradition, lupin seeds are preserved in brine (similar to olives) and served as an aperitif. They are also sprouted and used in salads [11].

Hippocrates, in his writings on human nutrition (460-377 BC), was said to have made particular mention of lupins as easy to digest without causing the flatulence characteristic of many other legumes. Galenus apparently detailed the debittering process as common practice, and Cato, in "*Di agricultura liber*"(200BC), referred to a "*labrum lupiniarum*", a special vessel used to remove bitter alkaloids from lupin seeds. Early mention was also made of the effectiveness of lupins in soil fertilisation. Palladius purportedly advised that plowing lupin into the soil had effects similar to using manure [12].

In the 1870s *Lupinus cosentinii* seeds were reportedly delivered to Port Gregory, north of Geraldton, as food for the convicts. In the 1950s *Lupinus angustifolia* was introduced from Europe into Western Australia [13]. Today, Australia is the world leader of sweet lupin production (*L. angustifolia and L. albus*), in 1999 producing 1365 x 10^6 kg [14].

2.1.2 RATIONALE FOR HUMAN CONSUMPTION

Existing intensive animal farming for meat production creates an unsustainable strain on the environment through excessive use of energy, space, raw materials and gaseous emissions [14]. Progress towards sustainable food production involves development of alternative sources of protein as meat extenders and replacements, as well as animal feed.

Seeds from the plant genus *lupinus* are high in crude protein (28-48%) content and low in anti-nutrient factors [14].

Lupin seed contains a similar crude protein level to soybeans, and about twice as much as any other legumes [10]. While most legumes are deficient in the sulfur amino acids, methionine and lysine, it has been shown that when rats' experimental diet (10% lupin) was supplementation with 0.1% DL-methionine, the growth rate and protein efficiency ratio (PER) was comparable with casein [10] [13].

In contrast to the soybean, the seed of sweet lupins, such as *L. angustifolia,* contain very low levels of anti-nutritive factors [2]. Typically, there are no lectins (phytohaemagglutinins); 0.14 mg/g (compared to 17.9 mg/g in the soy bean) trypsin inhibitors, which can disrupt digestion; 5.8 g/kg (0.44% compared with 1.59% in the soy bean) phytate and less than 0.02% tannins, both of which can bind with protein, iron, zinc and calcium thus reducing bioavailability of these essential nutrients [15].

The lupin seed is rich in beneficial oligosaccharides that have demonstrated prebiotic effects *in vitro* on bifidobacteria from the colon of rats [16]. The hulled fiber is associated with lowering cholesterol [14] and blood sugar levels [2], although these claims need further investigation [17]. The lipid fraction of the seed is reported to contain high levels of vitamin E [14]. The main fatty acid in *L. albus* is oleic (50%) and in *L. angustifolia* is linoleic (35-50%)[13]. The ratio of polyunsaturated fatty acids is similar to soy oil; 10:1 omega-6 to omega-3 [2]. It has also been reported to have a favorable unsaturated fatty acid to saturated fatty acid ratio of 6:1 [18].

2.1.3 LUPINS AS NOVEL PROTEINS

Novel proteins are old proteins that may have traditional uses in human consumption and animal feed, that are introduced for use in different ways. Lupin seeds may fall into this category as the bitter seeds have been used as food around the Mediterranean (*L. albus*) and in the Andes (*L. mutabilis*) for centuries. Although high in alkaloid levels that are poisonous in toxic doses, a lengthy "debittering" process, involving soaking and boiling

the seeds over days, leaches the alkaloids out into water to be discarded. The process detoxifies the seeds of the bitter-tasting alkaloids and prepares them for human consumption.

The naturally occurring low-alkaloid *lupinus spp* were thought to have been disadvantaged by the process of natural selection and, presumably due to consumption by animals, did not survive in the wild [12]. However, modern agricultural techniques, initially through selective breeding programs and more recently genetic engineering, have developed low-alkaloid strains, known as "sweet" lupins. These varieties are non-toxic, are an excellent source of nutrition, and apparently need not even be cooked before consumption [13].

There are increasing applications for lupins in Asia for miso, tempeh and tofu since lupin proteins have demonstrated higher yields of fermented products than soy [2]. In Europe, lupin flour is used to enrich protein and fiber content of bread, pastas, cakes, and biscuits [12]. Baking quality is not affected up to amounts of 10% (lupin to wheat flour) in bread and 50% in biscuits [2, 14]. Lupin products have a natural yellow colour that has been reported to enhance the aesthetics of lupin supplemented baked products [2]. A high level of lupin flour was reported to be a “good and safe” source of protein for young adults in Spain [19].

3 THE GENUS *LUPINUS*

The genus *Lupinus L.* (common name lupine or lupin) belongs to the subfamily Papillionaceae of the Leguminosae family of flowering plants [12]. There are 450 species [7], which incorporates 874 named members of the genus *Lupinus L.* [20]. Four species have been cultivated for animal and human consumption, which includes both “bitter” and “sweet” varieties of *L. angulstifolia* (blue or narrow-leaf lupin), *L. albus* (white lupin) and *L. luteus* (yellow lupin) from Europe, and *L. mutabilis* (pearl or tarwi lupin) from South America [21].

3.1 TOXICOLOGY

Natural toxicants enhance survival in plants, not least by serving as pesticides [22]. However, some of these compounds, especially the alkaloids, are capable of pharmacological effects in humans and animals. In sufficient quantities alkaloids can be poisonous, precipitating an acute toxicosis that may result in death. Alkaloid profiles and levels vary with the plant species and are influenced by environmental and genetic factors [23].

Although extensive toxicological research of lupin alkaloids on laboratory animals has been undertaken, a full review of this literature is outside the scope of this report. Interestingly, there appears to be marked between-species difference in the acute lethal dose of lupin alkaloids. For this reason the Australian New Zealand Food Authority advises that animal models are not suitable in the determination of a human lethal dose of lupin alkaloids [24].

3.1.1 LUPIN ALKALOIDS

There are many toxic alkaloids present in *lupinus spp*, including pyrrolizidine and piperidine alkaloids [25]. However, in the species of agricultural interest the toxic compounds of general concern, the quinolizidine alkaloids, are commonly referred to as "lupin alkaloids". This class of molecules is characterised by the presence of one or two quinolizidine rings in the structure. There have been approximately seventy different quinolizidine alkaloids identified in *lupinus spp*. Quinolizidine alkaloids are not limited to *lupinus spp* and have been found in many genres of the pea (*Fabaceae*) family as well as several other families [23]. The frequency and distribution of lupin alkaloids varies according to species. The major alkaloids in *L. albus* are lupanine, 13-hydroxylupanine, and some European varieties contain sparteine. The major alkaloids in *L. angustifolius* are lupanine, 13-hydroxylupanine, and angustifoline [13].

Both sparteine and lupanine have been shown to inhibit sympathetic nerve transmissions at the ganglionic level (ganglioplegic agents). They also inhibit preganglionic stimulation

of the vagus nerve [26], responsible for parasympathetic regulation of heart rate, breathing and digestive mobility [27]. Sparteine has been used as an antiarrhythmic drug and an oxytocic agent. However, in a clinical trial in the USA in the 1950s, sparteine was associated with death of the fetus due to over stimulation of the uterus and is therefore no longer used as such [28]. Both sparteine and lupanine exhibit binding power similar to nicotine for nicotinic receptors [26]**.**

3.1.2 ACUTE LUPIN ALKALOID TOXICOSIS IN LIVESTOCK

Lupinus spp reported as toxic to livestock, such as *L sericeus*, are high in quinolizidine alkaloids and are found in abundance to grazing animals [23]. Clinical signs of toxicity in animal studies are related to the total alkaloid content ingested [25]. The bitterness of high alkaloid lupin species and varieties usually deters animals from excessive grazing. However, many cases of livestock toxicosis from lupin alkaloids are documented. In severe cases there is a marked depression of respiration in the animal, sometimes convulsions and tremors, head butting, and wild thrashing of the body, before coma and death by respiratory paralysis [23]. Quinolizidine toxicosis resulting in death of farm animals occurs typically when large amounts of the podded lupin are ingested within a short time period [23]. This is rare in grazing cattle and horses but more frequent in sheep.

3.1.3 TETROGENIC EFFECTS OF LUPIN ALKALOIDS IN LIVESTOCK

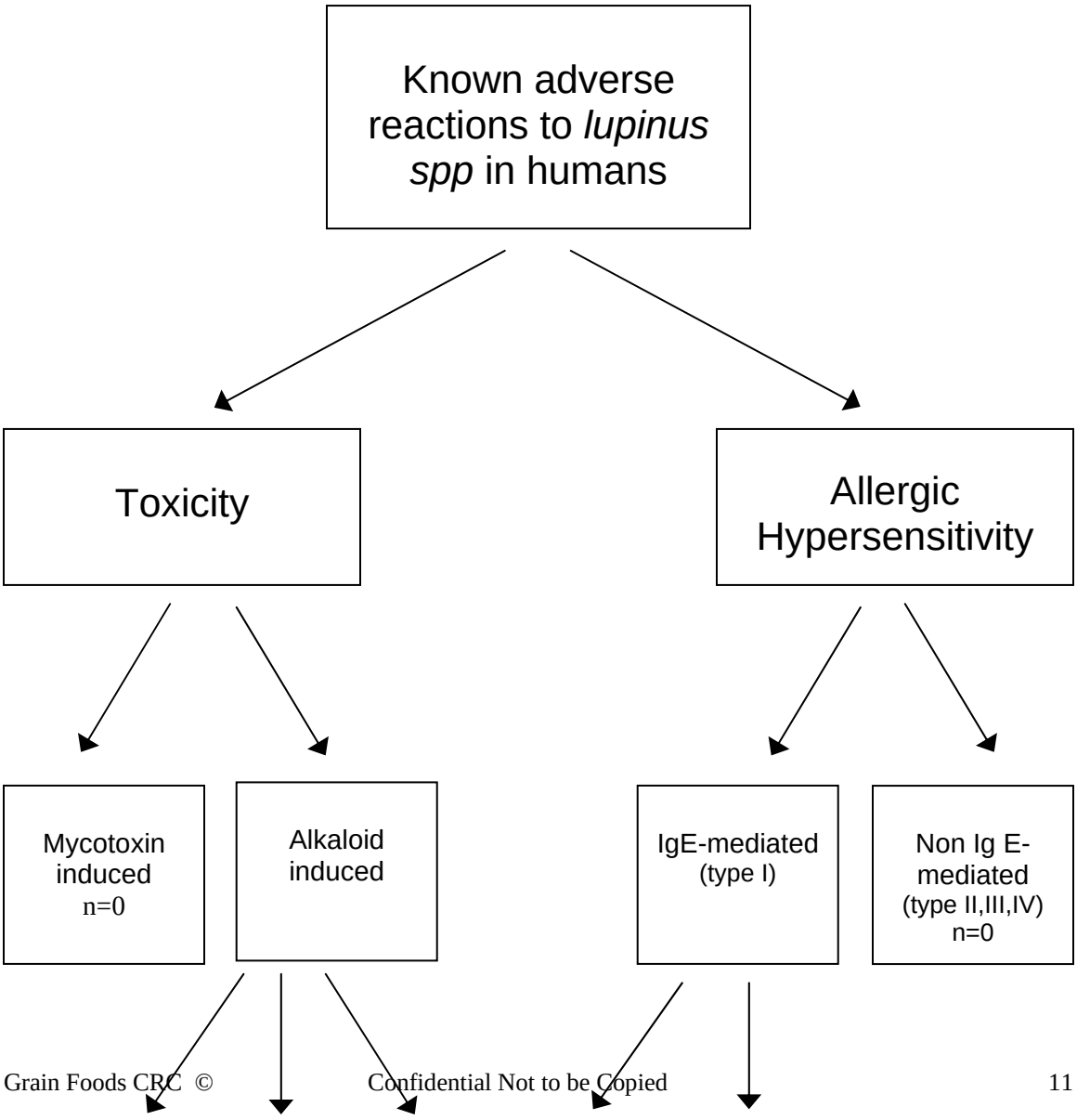
Although cattle rarely graze on lethal doses of high alkaloid varieties of lupin, some lupin species are tetrogenic, capable of inducing birth defects. Ingestion of sufficient quantities during critical times of maternal gestation produces "cooked calf disease", a syndrome characterised by serious and irreversible skeletal deformities in the calf. The active tetrogen was identified as anagyrine [23]. *Lupinus spp* commonly used as food for human consumption do not contain anagyrine [29]. However, Keeler [23] reports a study that detected anagyrine in the milk of lactating goats fed on anagyrine-containing lupin seeds, implicating the risk of cross species and human exposure to the tetrogen through goat's milk. Ammodendron, a piperidine alkaloid from *Lupinus formosus* and *L. arbustus* has also been linked with teratogenic activity in livestock [25].

3.1.4 LUPINOSIS

Another alkaloid-induced toxic syndrome observed only in farm animals is known as "lupinosis". The fungus *Diaporthe toxica*, which produces phromopsin toxins, colonizes sand-plain lupins, such as *L. cosentinii* grown in Western Australia, under certain conditions. The toxicity syndrome produced by these alkaloids involves liver damage and death of the grazing animals, usually sheep [30]. Fortunately, there have been no reported cases of lupinosis in humans [31].

4 ADVERSE REACTIONS TO LUPINS IN HUMANS

Adverse reactions to lupins are summarised in the flow diagram given in figure 1.



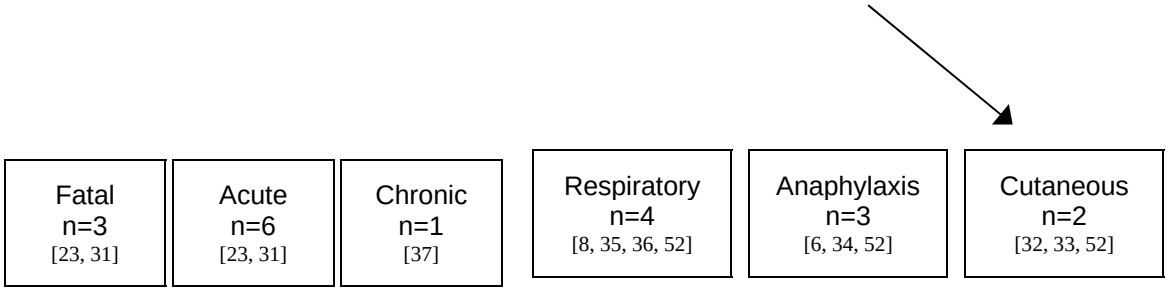


Figure 1: Summary of reported adverse effects of human consumption of lupins, showing number of cases reported categorised by clinical manifestations.

In the past, adverse food reactions to lupins in humans involved alkaloid toxicity. Toxicity depends on the amount of toxic alkaloids ingested. Isolated cases of lupin alkaloid poisoning in humans have been recorded. More recently there have been increasing reports of allergincity associated with lupin proteins. This may be due to increasing prevalence of food allergies coupled with increasing exposure to lupins.

4.1 LUPIN ALKALOID TOXICITY IN HUMANS

Intoxication by lupin seeds in humans is fortunately rare. A review of the literature [in German] prior to 1980 is reported [23] to have surmised that oral doses of various bitter lupins (3% mixed alkaloids) were lethal at 11-46mg/kg in three out of five (possibly children [31]) human subjects, and produced serious acute toxicosis in the other two subjects. Human toxicosis was reported to include malaise, nausea, visual disturbances, progressive weakness and respiratory arrest.

4.1.1 ACUTE LUPIN ALKALOID TOXICOSIS IN HUMANS

Lowen and Alam *et al* [31] reported the first case of lupin alkaloid poisoning in Australia. After ingestion of two handfuls of bitter tasting lupin beans with the evening meal and a smaller portion the following morning a 35 year old Mediterranean women presented to hospital emergency with an anticholinergic syndrome (inhibition of the nerve impulses of the parasympathetic nervous system [38]). Signs and symptoms included blurred vision, dilated pupils, tachycardia, dry mucous membranes, urinary urgency, muscular weakness

and exhaustion. She fully recovered after a night in observation on intravenous fluids and a urinary catheter.

It transpired that she had purchased some bitter lupin seeds (known in the Mediterranean as “lupini” beans), but had not adequately debittered the seeds before ingestion. Lowen and Alam *et al* [31] cite three child fatalities (possibly the same three cited in the German literature review) and four further cases of acute severe anticholinergic reactions due to lupin alkaloid poisoning.

A similar case was reported in the USA in 1999 [17]. A 72-year old Portuguese women ingested the water extracted after boiling bitter lupin beans. She said she believed it would lower her blood sugar levels. This was another clear case of anticholinergic syndrome; dilation of pupils, tachycardia, blurred vision and generalised weakness. Again the woman was discharged less than 24 hours later after making a full recovery with supportive care. The beans were found to be high in oxo-sparteine and sparteine.

4.1.2 PHENOTYPES IN METABOLISM OF LUPIN ALKALOIDS

In humans, the metabolic process of removing sparteine from the body involves the liver detoxification enzyme cytochrome P450-IID6 [17], which may be deficit in up to 10% of the population. Two phenotypes for sparteine metabolism have been proposed by Eichelbaum and Spannbrucker *et al* [28]; metabolisers and non-metabolisers. In a study of 360 normal volunteers found 18 (5%) were unable to metabolise sparteine, excreting almost 100% of the alkaloid in the urine. The authors speculated that the 2-3% of women that experienced adverse effects of sparteine in the 1950s USA trial on oxytocic effects may well have been non-metabolisers.

As a result of capacity-limited renal excretion, the alkaloid spends approximately three times longer in the serum and increases the risk of accumulation and side effects such as blurred vision, dizziness and headaches [17]. A slimming product containing lupinine (sparteine) prompted a letter to the editor of *The Lancet* [39] urging for the withdrawal of the product from general sale on the grounds that excessive doses by slow metabolisers -

which they estimated to be 9% of the population - could result in blurred vision, dizziness, headaches, nausea, diarrhoea, anorexia, cramps, circulatory collapse, respiratory arrest, and possible adverse effects on the gravid uterus.

4.1.3 CHRONIC EXPOSURE TO LUPIN ALKALOIDS IN HUMANS

There is a single case in the literature of the adverse effects of long-term exposure to lupin alkaloids [37]. After ingesting approximately 3g of lupin seeds (from Portugal) per month for eight years, a 28-year old woman, living in France, developed motorneuron disease. Signs and symptoms began with progressive difficulty writing and knitting, and progressed to right-sided dystonia, progressive weakness, dysphagia, and fasciculations of the tongue and limbs. Recovery began within two months of cessation of exposure to the seed, but twenty months later had not completely resolved.

Details regarding the species and variety of lupin and amount of alkaloids were not reported in detail. The authors mentioned that the seed contained 50 times more alkaloids than *L. luteus* or *L. angustifolius* and suggested that seeds were from *L. albus*. They supposed that long-term exposure to the alkaloids may have caused death of motor neurons.

4.2 ALLERGIC REACTIONS TO LUPINS

In order to facilitate a discussion on the lupin seed as a potential food allergen some terms commonly used in food allergy are briefly introduced.

4.2.1 THE LANGUAGE OF ALLERGENICITY

An *allergen* is a chemical substance that can trigger an allergic reaction [40]. Most allergens are proteins that enter the body from the environment, and are not intrinsically harmful [41]. *Allergenic* is the ability of a substance to induce an allergic reaction (by binding the antibody, IgE) [40]. Once identified, an allergen is classed as *major* or *minor* according to whether more or less than 50% of sensitised subjects experience an allergic reaction following exposure [42]. An *allergic reaction* is a pathological immune response

to an allergen to which the person has been previously exposed and has developed antibodies [38]. *Hypersensitivity* is the clinical term used to describe the process of prior sensitisation and subsequent immune dysfunction following exposure to an allergen. There are four types of hypersensitivity, numbered numerically according to the immunologic mechanisms involved [41].

Allergic food hypersensitivity usually manifests as an immediate (type I) hypersensitivity as it is usually mediated by the antigen-specific immunoglobulin E (IgE). Target tissues of the type I hypersensitivity response, found mainly in the skin, respiratory and gastrointestinal system, contain substantial amounts of mast cells. When mast cells degranulate they release histamine, a potent proinflammatory mediator [41]. *Cross-sensitivity* or *cross-reactivity* is when an individual that is sensitive to one allergen experiences hypersensitivity after an initial exposure to a structurally similar allergen from a different genus.

Anaphylaxis is an immediate Type 1 hypersensitivity reaction. It is a sudden and severe response occurring within minutes after exposure to an allergen to which the person has previously been sensitised. Localised anaphylaxis involves local inflammation of the affected area of skin. Generalised, or systemic, anaphylaxis may involve symptoms of itching, vomiting, abdominal cramps, vomiting, diarrhea and breathing difficulties. There is laryngeal edema in more severe cases, and extreme cases may result in circulatory collapse, respiratory distress, shock and death [41].

Skin prick tests are a common method used to measure an allergic reaction in a sensitised individual. The allergen is placed onto the skin and the diameter of the wheel and flare reaction indicates the degree of severity to that allergen. *Radioallergosorbent (RAST) tests* quantify circulating levels of specific IgE antibodies *in vitro* [41]. The *oral challenge*, or randomised, placebo controlled, double blind, food challenge (RPDBFC) is the “gold-standard” measure of food allergy [40, 43]. An oral challenge should, however, be conducted under medical supervision due to the risk of generalised anaphylaxis in a sensitised individual.

4.2.2 CROSS REACTIVITY IN PEANUT SENSITIVE SUBJECTS

The first case of lupin allergy was reported by Hefle and Lamanske *et al* [33] in 1994 in the US, and involves cross reactivity. After ingestion of lupin-fortified pasta, a five-year old girl presented with skin eruptions (urticaria and angiodema). The girl had a known peanut sensitivity, which led the researchers to investigate a population of peanut sensitive subjects to determine the incidence of cross reactivity. The subjects were seven adults with history of anaphylaxis, positive skin pricks and RAST tests to peanuts. Five of the seven subjects tested positive in the skin prick test (SPT) to the lupin-fortified pasta.

The skin prick tests differed in severity and the RAST scores varied, demonstrating that there is only partial cross reactivity between lupins and peanuts. Interestingly, all the subjects returning positive SPTs also had green pea sensitivity. That is, cross-reactivity to other members of the legume family already existed in these subjects. The researchers claim this provides evidence for a substantial cross-reactivity to lupins in peanut allergy, especially in pea sensitive populations.

A more recent French study (1999) confirmed the high risk of cross reactivity in peanut sensitive subjects [7]. Skin prick tests of 11 out of 24 (44%) peanut sensitive adults were positive to lupin flour (*L. albus*). RAST tests indicated closely related allergens in lupin flour, lupin pollen and peanuts. Most interesting, immunoblotting tests demonstrated that the major allergen in lupin flour had the same molecular mass (43 kd) as that found in peanut.

These findings, however, must be tempered by methodological considerations as discussed below in section 3.2.7. Basically, results obtained by immunochemical measures are rarely correlated with clinical manifestations of allergy. Only with an oral food challenge can a clinically important food allergy be correctly diagnosed [43].

4.2.3 LUPIN ANAPHYLAXIS

A 38 year old woman with a history of atopy presented with urticaria, angioedema, shortness of breath and laryngeal edema immediately after eating 3 lupin seeds in Spain in 1996 [34]. It was the first time she had tried lupins, however she had a history of allergic food hypersensitivity to other legumes.

In 1992, skin prick tests were positive to chickpea after she had investigated the cause of recurrent urticaria and angioedema. In 1995 she noticed wheals and throat swellings after eating lentils and white beans. Skin prick tests in 1996 were positive to chickpea, white bean, lentil, pea, soybean and lupin but negative to peanut and green bean.

An open oral challenge was performed with foods to which the patient was apparently tolerant. Immunoblotting tests confirmed the cross reactivity of some of the legumes, but there were discrepancies between the clinical tolerance to peanut and its IgE binding *in vitro*. This case highlights a progressive potential for allergic cross-reactive hypersensitivity.

In 2002, two children were hospitalised in a Pediatrics department in France, one for asthma and the other for meningeal syndrome [6]. A chocolate powder given for breakfast induced generalised anaphylaxis in both children. Although it did not appear on the label, investigation of the product revealed the presence of lupin flour. Both children had histories of peanut allergy and skin prick tests confirmed a cross sensitivity between lupin and peanut. This case highlights a potential of lupins to become hidden food allergens, if processed foods containing lupins are not labeled as such.

In Australia there have been a small number of reported reactions to lupins [52]. This has included two women who appear to have experienced anaphylactic reactions to products containing lupins and a third experiencing respiratory problems. Of the two women reported to have anaphylactic reactions, one was tested for her allergenicity to soy and peanuts and was found to be negative. Both women showed strong positive reactions to skin prick tests performed using a saline extract of lupin bran.

4.2.4 RESPIRATORY SYMPTOMS AFTER INHALATION OF LUPIN DUST

Inhalation of lupin dust precipitated a severe allergic asthma attack in a three-year old boy with a history of episodic asthma in Italy [35]. He had been playing near a tree that had been fertilised with lupin dust. He presented twice to a clinic with severe asthma accompanied by sudden onset of conjunctivitis, rhinorrea, cough and cyanosis.

Multiple skin prick tests included peanut, lentil, bean and chickpea were all negative. Positive responses to grass pollen and *Dermatophagoides pteronyssinus* (mite) were reported. There was also a marked positive response to lupin seeds. A provocation test using the actual dust was performed and within 3 minutes the respiratory symptoms returned. RAST and immunoblotting tests with the dust confirmed the IgE binding, but also showed a cross reactivity with an allergen in the peanut. It is interesting to note the discrepancy between SPT and immunoblotting with regards to peanut reactivity. Similar with that found by others [33], immunoblotting found a molecular weight of 45 kd for the major IgE binding protein in lupin.

4.2.5 OCCUPATIONAL ALLERGY TO LUPIN FLOUR

A 30-year old female environmental technician presented with repeated episodes of rhinitis and conjunctivitis in France [36]. She did not have a history of atopy or peanut allergy. Her job involved handling lupin flour for skin prick tests and oral food challenges. Skin prick tests were positive for grass pollen (5mm) and *Dermatophagoides pteronyssinus* (3mm). Prick tests with lupin flour both raw and cooked were positive (8mm). RAST tests were positive to lupin flour and peanut but not to lupin pollen. She ceased handling the lupin flour and her symptoms had not returned on follow-up six months later.

In a study on occupational exposure to lupin flour by Crespo and Rodriquez *et al* [8] seven subjects working at an agricultural research center in Spain were studied for employment history, work related symptoms and potential risk factors. Skin prick tests with *L. albus,* serum tests for IgE binding, bronchial provocation tests for subjects reporting respiratory symptoms, and a double blind, placebo controlled food challenge.

Three subjects reported respiratory symptoms with an onset of 5 minutes to one hour after commencing work with lupin flour. Skin prick tests were also positive in these subjects. However, IgE binding was detected only for two subjects. Immunological cross reactivity to other legumes, bronchial provocation and DBPCFC were also positive in these subjects. On bronchial provocation with lupin flour, one subject had a 25% reduction in FEV_1 (forced expiratory volume in one second, a pulmonary function test) while the other had naso-ocular symptoms without FEV_1 changes. This study demonstrated that it is possible for workers handling lupin flour to become sensitised to lupin via the respiratory system, which may subsequently lead to occupational asthma and food allergy.

4.2.6 CONTACT URTICARIA FROM LUPIN

A 25-year old male with a history of seasonal rhinitis reported onset of urticaria when kissing his girlfriend in Spain [32]. She had previously been eating lupin seeds. The wheals and severe itching were restricted to the contact area. In skin prick tests the subject was positive to grass pollens (*Olea europeaea* and *chenopodium album*) and reported mild pruritus (itching) of his lips and mouth after ingestion of lupin seeds years previously. Skin prick tests for lupin was negative for dried and fresh lupin, but positive for wet lupin (diameter 8mm within 15 minutes). The tests were then repeated on healthy volunteers and 10 atopic individuals but were all negative.

4.2.7 METHODOLOGICAL ISSUES PERTAINING TO ALLERGY DIAGNOSIS

Studies that rely on skin prick tests and/or *in vitro* IgE binding in order to diagnose clinically relevant allergenicity or cross sensitivity are methodologically flawed. While they may be useful in ruling out food allergy (true negative), the major problem with SPTs is they yield a high rate of false positives. Therefore, a positive skin prick test does not accurately or reliably predict clinical hypersensitivity.

Bernhisel-Broadbent and Sampson [43] demonstrated in sixty nine children with legume hypersensitivity that had undergone skin prick tests and oral food challenges, that in the case of positive SPT to peanut only 52% were confirmed with an oral challenge, and in the case of soy, 33% were confirmed. That is, 67% of positive skin prick tests for soy were in fact false positives.

The authors acknowledge that SPTs are useful in the case of negative results confirming that the person does not have hypersensitivity to the allergen, but stress the importance of the oral challenge in confirming a diagnosis of food allergy. The only exemption from this procedure would be the case of a clear history of anaphylaxis to that allergen. Then the history is taken in lieu of the oral challenge.

When diagnosis was contingent upon confirmation by oral challenge or history of anaphylaxis, the frequency of cross reactivity among legumes in a pediatric population in France was estimated to be 5%. These findings are in accord with other observations that cross recognition between legume allergens are ‘immunologically frequent but clinically rare’ [44, 45].

5 SUMMARY OF THE FOOD SAFTEY ISSUES RELATING TO HUMAN CONSUMPTION OF LUPINS

The World Health Organisation describes *food safety* as food that does not cause harm when consumed in the manner intended for its use. Traditionally, food safety regulators have been concerned with toxicology, infestation, and nutritional and anti-nutritional aspects of foods [46]. In the case of lupins, the high levels of alkaloids in the bitter species and varieties have caused toxicosis in two cases published since 1980. Lowen and Alam *et al* [31] suggest that labeling of the bitter varieties, with accompanying instructions on the correct method of de-bittering would prevent any further cases of accidental lupin toxicosis.

The rising prevalence of food allergy, however, introduces another major safety concern. Between 1-2% of adults [2, 47, 48] and up to 8% [6] of children are reported to have an

IgE-mediated food allergy. The prevalence of food allergy is increasing (currently 3.4% in France), perhaps as a result of compound environmental factors such as disruptions of intestinal microflora [5], lack of exposure to viral and bacterial infections in childhood [49], and changes in weaning practices [50].

More than 160 foods have been reported to induce IgE-mediated reactions [46]. New allergens are frequently being identified [51]. However, conclusions are often drawn from case reports or results of skin prick tests and positive *in vitro* tests for IgE binding [45]. This approach is problematic as there are significant and substantial discrepancies between skin prick tests, IgE positive results and clinical manifestations of allergy. Reporting positive results in immune function tests without validating the findings with clinical manifestations elicited from an oral challenge has the potential to obscure the actual incidence and severity of the progression of food allergy.

The way in which a food allergy evolves in the community is largely unknown [9]. Some of the most common proteins are now major allergens, such as milk, wheat, peanuts and soy. Currently, the only prophylaxis for individuals with food allergy is the avoidance of all foods containing the allergen [41]. Correct labeling of food allergens in packaged or processed foods is an important safety requirement for this population. Warner [9] has suggested post-market surveillance of potential allergens such as those found in lupin, in order to minimise the time between detection of a problem and the implementation of appropriate action.

5.1 ASSESSMENT OF ALLERGENICITY FOR NOVEL AND GM PROTENS

Rigorous protocols are applied in the assessment of the allergenicity of a new protein, such as a novel protein or a protein from genetically modified (GM) crops, prior to its release onto the market. The application of these protocols to existing allergens may bring clarity to the study of food allergy, its clinical relevance, severity and prevalence.

Essentially, when a novel and/or genetically modified protein is suspected of containing major and/or minor allergens, the sera of at least 14 sensitive subjects are required (at

95% significance) for solid phase immunology. If this quantity of sera is not available, then results from tests on available sera are used in conjunction with other laboratory tests regarding the stability of the allergen after exposure to digestive enzymes, such as hydrochloric acid, and heating. Results from the laboratory tests are correlated with skin prick tests. Findings in SPTs are verified with a double blind, placebo-controlled, food challenge (DBPCFC). If there are any positive findings in the solid based immunoassay, the skin prick tests, or the DBPCFC, then it is recommended that food labels contain a note on the source of the allergen [40].

5.2 ASSESSMENT OF THE ALLERGENICITY OF LUPIN

The allergenicity of lupin is difficult to determine from the literature. The results obtained by Helfe and Lemanske *et al* [33] suggest that lupin is a major allergen in peanut sensitive subjects, as 71% of subjects and 100% of peanut and pea sensitive subjects returned positive skin prick tests to lupin. However the clinical relevance of these findings cannot be extrapolated without an oral challenge (DBPCFC).

Moneret-Vautrin and Guerin *et al* [7] demonstrated positive skin prick tests in 11 of 24 peanut sensitive subjects (44%) and a sub-group of 6, where 6 (100%) demonstrated positive oral challenge to lupin. Crepso and Rodriguez *et al* [8] found that 2 of 7 (29%) workers investigated developed lupin hypersensitivity after occupational exposure to lupin flour. Moreover, 1 of 7 (14%) developed food allergy to lupin after being sensitised through inhalation of lupin flour.

To date the reports of lupin allergenicity are largely anecdotal. However, there is evidence that lupin has moderate to high allergenic potential. Petterson [2] suggests that the allergenicity of lupin is similar to peanut, arguably the most common known allergen [45]. Lupin pollen seems to have higher allergenicity than the flour or seeds, prompting some to hypothesise that the presence of non-allergenic proteins reduces the coupling of allergenic proteins with IgE [7]. While the allergens in lupin appear to be heat stable

[44], some food processing such as fermentation has been shown to substantially reduce allergenicity, as demonstrated in soy products [8].

5.3 CONCLUSION

There are increasing reports of allergic reactions to lupins, especially in peanut sensitive individuals. In this population there have been three cases of lupin-induced anaphylaxis; two cases were young children in hospital that reacted to a hidden ingredient in a chocolate drink provided for breakfast. Children, atopic- especially peanut-sensitive - individuals, and people occupationally exposed to lupin flour appear to be most susceptible to lupin hypersensitivity. It can only be envisaged that as exposure to lupins is increased that there will be a corresponding increase in reported cases of lupin induced hypersensitivity and anaphylaxis.

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7 APPENDIX

7.1 SEARCH TERMS, DATABASES AND REFERENCE MANAGERS.

Data base searches were conducted 23 Feb 2004 - 9 March 2004. Search terms used were lupin*; hypersens*; adverse; safety; consum*; allerg*; anaphyl*; cross react*; Unilateral searches were conducted in multiple databases, followed by combined searches. For example lupin* hypersens*.

Databases searched include Pubmed; Medline; AGNR:ABOA (Agriculture); AGRICOLA; AgDB (National Agricultural Library's Directory of Agriculture-Related Databases, Datasets, and Information Systems); AgNIC (Agriculture Network Information Centre); Agrisurf (Grains Research and Development Homepage); CAB (Agriculture); CABDirect (Nutrition); CD Biological Sciences Library UQ: Proquest 5000 (Proquest); AMED (Allied and Complementary Medicine OVID); Blackwell Science and Wiley Interscience; CINAHL (Nursing and Allied Health Literature OVID); Expanded Academic Index (Infotrac).

An internet search using the search engine GOOGLE and the search terms "lupin human consumption adverse effects" identified the paper on toxicology from the ANZFA (Australia New Zealand Food Authority) webpage. Other webpages searched include WHO (World Health Organisation) and CSIRO (Commonwealth Scientific Industrial Research Organisation).

References were managed in Reference Manager 7 software.