

LUPIN ALKALOIDS IN FOOD
A Toxicological Review and Risk Assessment

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SUMMARY

Quinolizidine alkaloids are found in various plants including those belonging to the *Lupinus* genus although the nature and level of these alkaloids is highly variable between species. While they are not the only alkaloids found in lupins, they are the major concern in relation to human and animal health.

The levels of alkaloids in seeds or meal can be reduced through a de-bittering process involving soaking or washing with water. This is commonly practised in Europe where high alkaloid lupins, so-called 'bitter lupins', are grown. The level of alkaloids in these lupins after the de-bittering process is reported to be approximately 500 mg/kg. In Australia, lupin varieties with low alkaloid content, so-called 'sweet lupins', have been developed through plant-breeding programs. Data indicates that the mean alkaloid content of marketable sweet lupin seed is on average 130-150 mg/kg.

Lupin alkaloids may be found in any derivative of the seed or plant, including flours and meal that can be used to prepare pastas, pastries and dairy product substitutes. Traditionally, lupin seeds of *Lupinus albus* following debittering have been used in the Middle East and Europe to make snack foods. In Europe, lupin seeds are known as lupini beans. Lupins are also used in traditional fermented foods such as tempe, miso and soy sauces in Indonesia and Japan. More recently, lupin derivatives are increasingly being introduced into food for human consumption through the use of lupin flour derived from low alkaloid varieties.

Hazard assessment

Little data is available on the metabolism and excretion of alkaloids in animals or in humans. In one human study, orally administered lupanine was excreted unchanged in the urine with a half-life of 6-7 hours.

The majority of the acute studies have been performed on the common lupin alkaloids, sparteine and lupanine. Both have moderate acute oral toxicity in rodents although sparteine is the more toxic. The symptoms observed suggest the alkaloids cause toxicity through neurological effects leading to loss of motor co-ordination and muscular control. The effects are generally reversible.

In 3-month feeding studies in rats, there was little evidence of toxicity, even at the high dose levels. At a dose level of 505 mg/kg bw/day, haematological changes were the only significant effect that could be linked to treatment. The no-observable-effect level (NOEL) for these studies was in the range 90-105 mg/kg bw/day.

A two-generation reproduction study in rats revealed no adverse effects on fertility, lactation or any other reproductive parameters at a dietary level of 12 mg/kg bw/day, which was the only dose level used.

In a study to investigate the neurotoxicity of lupin alkaloids, sparteine and lupanine when administered by intravenous route inhibited ganglionic transmission of the sympathetic nervous system. Lupanine also suppressed the effects of pre-ganglionic stimulation of the pneumo-gastric nerve in the parasympathetic nervous system. This study may provide some insight into the mechanism of acute toxicity.

There are no chronic studies available that specifically examine the toxicity of lupin alkaloids, however, rats fed a low alkaloid lupin seed-based diet did not show any evidence of toxicity after 2 years.

Human acute toxicity studies were restricted to anecdotal reports of poisoning cases. General toxic symptoms included malaise, nausea, respiratory arrest, visual disturbances, ataxia, progressive weakness and coma. On the basis of the data available, the acute lethal dose for humans is approximately 30 mg/kg bw, which is considerably lower than the lethal dose levels reported in rodents.

The only data available on human chronic toxicity are the reports of traditional use of lupini beans in Europe, which indicate a daily dose of 0.35 mg/kg can be tolerated in adults without adverse effects. On the basis of this limited data, however, it is not appropriate to consider this dose level as the safe level for all individuals in the population. The only data available on the levels of alkaloids in lupini beans is anecdotal – there seems to be no published information available. Also, the information applies only to adults, not children, and it is likely that the adult population has developed a certain amount of tolerance to these alkaloids. The limited metabolism data available, however, suggests that the alkaloids are rapidly excreted unchanged which would reduce the likelihood of chronic toxicity.

If a safety factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake (PTDI) for humans is 0.035 mg/kg/day or 35 µg/kg/day.

Dietary Exposure Assessment

There are no dietary survey data available from which to determine food consumption levels of lupin alkaloids since lupins currently have very limited use in foods. Based on conservative assumptions regarding the potential for use of lupin flour in wheat-based products and the typical concentration of alkaloids in lupins harvested for human consumption, the data indicates that consumers of products such as pasta, pastry and cakes and biscuits would be likely to have a daily exposure to lupin alkaloids of 2 µg/kg bw/day at the 95thile of consumption.

The level of exposure to alkaloids from home use of lupin seeds is difficult to assess. The European experience suggests that lupin seeds in the home are most likely to be consumed as a snack food. Low alkaloid varieties of lupin seeds contain approximately one quarter of the alkaloid content of debittered European lupini beans and thus are unlikely to cause symptoms of toxicity for the majority of the population. However, given the paucity of data, it is not possible to state that ingestion of these lupins will be without adverse effects for all individuals in the population.

There is little information available on the effect of heating or cooking on the stability of lupin alkaloids although they are known to be soluble in water as shown by debittering processes.

Risk Characterisation

The available data on lupin alkaloids is limited and does not allow a full characterisation of the risk of exposure to humans. It is of particular concern that the available data indicates that humans are more susceptible to the toxicity associated with lupin alkaloids than other species. The traditional use of debittered lupini beans in Europe as a snack food has been reported to be without adverse effects at a dose level of 0.35 mg/kg/day in adults. On the basis on this data, a tolerable level of exposure for humans has tentatively been established at 35 µg/kg/day using an uncertainty factor of 10 in order to account for the variability in the human population.

The major potential source of exposure to lupin alkaloids is the use of lupin flour from low alkaloid varieties of lupins to substitute for a small percentage of wheat flour. The available information on potential exposure via lupin flour suggests that at current levels of use, human exposure will be well below this tolerable level of exposure.

There is also potential for exposure to these alkaloids through the use of lupin seeds as a snack food. At present, this practice is uncommon and confined to a sub-population of southern European immigrants. Given the uncertainty regarding the toxicity of lupin alkaloids, there may be cause for concern if this practice were to become commonplace, particularly if lupini beans (the large seeded bitter cultivars of *L. albus*) were to be widely marketed. The consumption of the lupini bean in Australia and New Zealand is currently not a concern since the current consumers understand the de-bittering process required. While the low alkaloid varieties grown in Western Australia have approximately one quarter the alkaloid content of de-bittered lupini beans, on the basis of the data available, it is not possible to state that ingestion of the low alkaloid varieties will be without adverse effects for all individuals in the population.

In order to characterise further the potential human risk associated with lupin alkaloids, additional research is required to establish the basis for the observed toxicity in humans.

INTRODUCTION

Quinolizidine alkaloids are found in various plants including those belonging to the *Lupinus* genus (family *Leguminaceae*). These lupin alkaloids are considered poisonous at high levels - recognised to be 1-2% alkaloid concentration in the plant (Butler, et al, 1996). Levels and combinations of alkaloids are highly variable between plant species - the alkaloid profiles of domesticated *Lupinus* species is shown in Table 1. These alkaloids are not restricted to the *Lupinus* genus, being also found in several members of the pea family. Quinolizidine alkaloids are not the only alkaloids found in lupins but they represent the greatest concern. There are almost 70 different quinolizidine alkaloids found in various *Lupinus* species.

The notable alkaloids of interest in human and animal health are:

- lupanine (a ketonic derivative of sparteine) and its isomers;
- sparteine - mainly isolated from broom but also present in several species of *Lupinus*;
- anagyrine - a teratogenic alkaloid, which is not present in the major cultivated species for human consumption (James and Keeler).

Lupins are often referred to as either bitter or sweet. Bitter lupins, such as the lupini beans consumed in Europe, have high concentrations of alkaloids (mainly sparteine), which make them bitter to the taste, and a debittering process is required before consumption. Sweet lupins, such as those grown in Western Australia, have low levels of alkaloids (mainly lupanine).

Other alkaloids that are of concern belong to the group of piperidine alkaloids (James and Keeler, undated). These are found in a range of other plant genera (eg. *Piper* spp), but are also peculiar to the lupin species *L. formosus* and *L. arbustus* which are pasture species found in the USA. These alkaloids are suspected to be teratogenic as well as causing general toxicity (Panter et al, in Garland and Barr, 1998) but are not found in domesticated species.

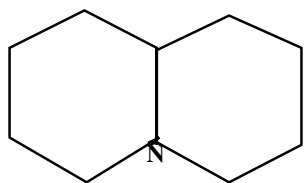
Table 1. Quinolizidine alkaloids in the major lupin species (% of total alkaloids)¹

Alkaloid	L. Albus	L. Agustifolius	L. luteus	L. Mutabilis
Albine	15	-	-	-
Lupanine	70	70	-	46
Multiflorine	3	-	-	-
13-Hydroxylupanine	8	12	-	12
Angustifoline	1	10	-	1
Lupinine	-	-	60	-
Sparteine	-	-	30	16
Ammodendrine	-	-	-	2
Tetrahydrohomifonine	-	-	-	2
3-Hydroxylupanine	-	-	-	12
13-Angeloyloxylupanine	-	-	-	2

¹. Adapted from Wink et al. (1995)

Chemical Properties

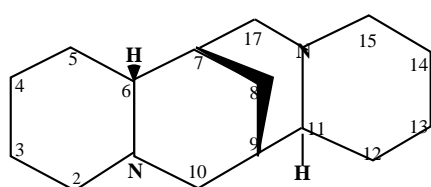
As a group, the quinolizidine alkaloids are based on a bicyclic Q ring system (see figure 1) and include bicyclic, tricyclic, tetracyclic quinolizidine alkaloids and up to 5 - 10 complex ring systems.



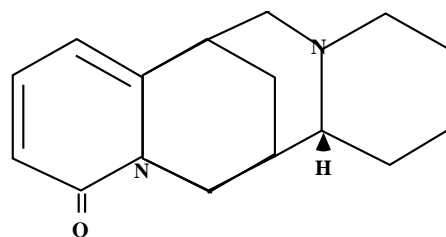
Quinolizidine

Figure 1: The basic quinolizidine structure

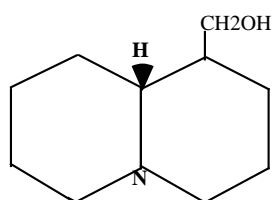
Alkaloids, which are peculiar to lupins, are restricted to bi- tri and tetracyclic examples (see figure 2). The lupin quinolizidine alkaloids differ from one another by the nature and pattern of substituents and configuration.



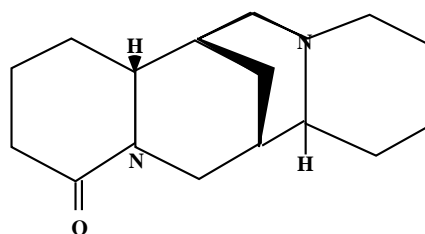
Sparteine



Anagryne



Lupinine



Lupanine

Figure 2: Some common lupin quinolizidine alkaloids

The piperidine alkaloids are composed of a piperidine ring with an attached structure of three carbons or more, located next to the ring. This structure is said to be required for teratogenicity. One particular piperidine alkaloid of concern to human health, found in lupine species - *L. formosus* and *L. arbustus*, is known as ammodendrine (see figure 3; Keeler and Balls 1978, in Garland and Barr).

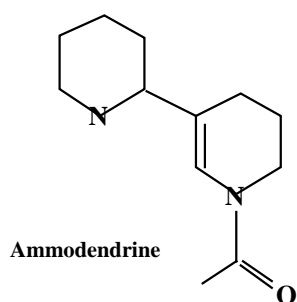


Figure 3: Chemical structure of ammodendrine

Sources of Exposure

Lupins are a good source of protein and lipids and offer the advantage of having no lectins and a very low content of protease inhibitors. There is utilisation of lupin seeds in many countries around the world - food products range from whole beans (eg, lupini beans which are the bitter cultivars of *L. albus* grown in southern Europe and the Andean lupin or tarwi of South America), to mashed beans and yoghurt and milk substitutes. Flours and meals derived from these products could be expected to contain alkaloids. Lupin bean meal is often used as a substitute for soybean meal (Pettersen, 1998). In the future, the most likely use for lupins in human food is as a base for fermented foods, such as Indonesian tempe and tauco. There is also potential for the use of lupin fibre in processed foods such as breads and baked goods. While there has been some interest in Australia in supplementing white and wholemeal breads with up to 10% lupin flour, there are currently no products on the market (Pettersen, 1995).

During the period 1982-1985, the mean alkaloid content of marketable sweet lupin seed was reported as 0.015% (150mg/kg) (Butler et al, 1996), and for the period 1985-1992 to be 0.013% (130mg/kg) (Robbins et al, 1996). These are below the current maximum permitted concentration (MPC) of 200mg/kg. The quinolizidine alkaloids are likely to be found in any derivative of the seed, including: flour, meal, pastas and pastries, dairy product substitutes, and coffee substitutes based on lupin seeds (Cheeke, 1989).

In Europe, the levels of alkaloids in the locally grown seeds or meal are reduced through debittering processes (using extract solvents) or by washing with water. The use of low alkaloid varieties is generally not feasible in Europe.

In Australia, plant breeding programs have focused on crop optimisation of species which have naturally low levels of alkaloids, as well as on the hybridisation of species with low native levels of alkaloids. Low alkaloid varieties have been available for a number of years and include *L. albus*, *L. augustifolius*, and *L. luteus* cultivars (*L. luteus* is a known source of sparteine) (James and Keeler, undated).

REVIEW OF TOXICITY DATA

Metabolism and Kinetics

There is generally little data on the metabolism, accumulation and elimination of alkaloids.

In a report by Peterson et al (1994), 11 volunteers were administered a single dose of 10 mg of lupanine or 13-hydroxylupanine in a gelatin capsule. The volunteers included both CYP2D6 extensive metabolisers and poor metabolisers in order to examine whether the genetic polymorphism that controls N-oxidation at the 1-position on the molecule could be a factor in the toxicity of lupin alkaloids (Eichebaum & Gross, 1990).

In all subjects, >90% of both lupanine and 13-hydroxylupanine were recovered unchanged in the urine. There was no evidence of any conjugation. The half-lives for excretion were approximately 6-7 hours. The data suggests that metabolism is not required for excretion of these compounds. The short half-life also reduces the possibility of systemic toxicity.

Acute Toxicity Studies

Acute toxicity studies in rodents are summarised at Table 2.

Symptoms of acute poisoning in mice are similar for IP and PO administration: trembling, toni-clonic spasms and death by respiratory arrest (Yovo et al, 1984). This has been attributed to a paralysis of breathing centres and inspiratory muscles. Other symptoms associated with lethal oral dose of alkaloid in all animals were tremors, splaying and paddling of the hind limbs within 2 to 16 min, convulsions, cyanosis and collapse. Death occurred within 2-23 min of administration of lethal dose. Two rats remained in collapse state for 2 and 4h before death (Pettersen et al, 1987).

Rats given lethal IP injections of pure alkaloid displayed neurotoxic signs within 1-13 min and died within 6-26 min (one rat died after 73 min). Response was identical to those of PO administration. Post-mortem examination showed congestion of liver and lungs. Few signs were observed in survivors and no further clinical signs of toxicity were observed. Rate of body-weight gain was the same as for untreated rats in the weeks following the study (Pettersen et al, 1987).

Although symptomatically similar, sparteine intoxication occurs at significantly lower levels than for lupanine in single dose parenteral or oral administration. This is supported in other literature (Robbins et al, 1996). There is notable difference in the LD₅₀ values of sparteine and lupanine in the mouse, though this is more marked when comparing levels for IP administration. The relative difference was confirmed in the guinea pig. In the rat it is apparent alkaloids administered IP have a lower LD₅₀ than after PO administration. LD₅₀ for mixtures of alkaloids are much higher when compared to those of pure lupanine (PO administration) and sparteine. The symptoms of acute toxicity indicate neurological effects, especially loss of motor co-ordination and muscular control.

Table 2. Summary of acute toxicity study results

Species and type	Route	Alkaloid	LD ₅₀ (mg/kg)	MMD* (mg/kg)	Reference
EOPS Swiss mice ¹	IP	Sparteine	36		Yovo <i>et al.</i> , 1984
"	PO	Sparteine	220		"
"	IP	Lupanine	175		"
"	PO	Lupanine	410		"
Hartley Guinea-Pig ²	IV	Sparteine		27	"
"	IV	Lupanine		78	"
Wistar rats (fed) ³	PO	Mixture	2279	2000	Petterson <i>et al.</i> , 1987
Wistar rats (starved) ³	PO	Mixture	2401	2600	"
"	PO	Lupanine	1664	1538	"
"	IP	Lupanine	177	154	"
"	IP	hydroxylupanine	199	169	"
129/AoBoy/Iiw mice ¹	Gavage	A ₁	820		Stobiecki <i>et al.</i> , 1993
"	"	A ₂	1900		"
"	"	A ₄	2050		"
"	"	A ₅	750		"
"	"	all other fractions	>4000		"

*MMD denotes Minimum Mortal Dose

1. The LD₅₀ of sparteine and lupanine was investigated in groups of ten EOPS Swiss mice per dose level in a study by Yuve et al. (1984). The ip doses were 30-42 mg/kg (sparteine) and 100-300 mg/kg (lupanine) and the po dose were 40-480 mg/kg (sparteine) and 175-700 mg/kg (lupanine).
2. Five Hartley guinea-pigs per dose level were treated using perfusion into the jugular vein (intravenous - IV). Minimal mortal dose (MMD) was determined in the Guinea-pig. Sparteine was perfused at the rate of 2mg/0.5ml/min and lupanine at 5mg/0.5/min, repeatedly until breathing arrest occurred (Yovo et al, 1984).
3. Groups of five male Wistar rats per dosage level were fed a diet consisting of a mixture of major alkaloids of *Lupinus angustifolius* (Petterson et al, 1987). The alkaloid "cocktail" consisted of 49% lupanine, 39% 13-hydroxylupanine, 10% angustifoline, 0.7% α -isolupanine and traces of other alkaloids. The individual alkaloids lupanine and hydroxylupanine were also tested. This study was limited in the data presented for interpretation.

The major alkaloids such as lupanine are said to have similar pharmacological properties to those of sparteine (Robbins et al, 1996, Butler, et al, 1996). However, the other alkaloids display different properties and may have different mechanisms of toxicity. Acute sparteine intoxication is characterised by neurotoxic effects such as decreasing cardiac contractility, ganglioplegia (blocking of ganglionic transmission) and oxytocic properties (contraction of uterine smooth muscle) (Yovo et al 1984, Robbins et al, 1996). For the alkaloid lupanine, observed properties are very similar to those of sparteine. This is confirmed in the above acute toxicity studies.

Though the data are not extensive, the differences in LD50s between alkaloid mixtures and single alkaloids appear to indicate that lupins contain other substances which inhibit toxicity, or that alkaloids inhibit each other in some way when in combination (as reflects the natural state). This was observed in the study using Wistar rats where the substances, lupanine and hydroxylupanine, were more toxic individually than when mixed.

Sub-chronic Toxicity

In a study by Ballester et al (1980), three groups of twelve 21-23 day old Charles River rats were given a diet containing alkaloids at the level of 0, 50 and 90mg/kg bw/day in a feeding study over 112 days. The three diets consisted of casein (control group), *Lupinus albus* (0.05% alkaloids, ie, 50mg/kg bw/day) and *L. luteus* (0.09% alkaloids, ie, 90mg/kg bw/day).

Food and water were available *ad libitum*, diet intake and body weights were recorded weekly. At the end of the experimental period weights of livers, kidneys, spleens, hearts and adrenals were recorded. Tissue samples of livers, kidneys, and lungs were microscopically examined.

Animals of both sexes fed alkaloids at the level of 90mg/kg bw/day gained weight at about the same rate as the control group, while those fed at 50mg/kg bw/day gained weight at a slightly lower rate, which was not statistically significant. There were no differences between intakes and feed efficiencies of lupines.

No differences were observed in organ-to-body weight ratios of any organs surveyed in any animals. At the end of the treatment period gross autopsy and histopathological examinations did not reveal any significant differences or cell alterations.

Summary - No significant toxicological effects were observed when rats were fed a diet containing up to 90mg/kg bw/day lupin alkaloids.

In a combined subchronic and reproductive study by Ballester et al (1984), two generations (F₁ and F₂) of groups of 20 male and 20 female weanling Wistar rats were fed diets based on sweet lupin flour containing 41.8% protein and 0.025% lupanine for nine months. Three comparable batches were prepared to give a diet containing 20% lupin protein, and alkaloid level of 0.012% alkaloid/diet (equivalent to 12mg/kg bw/day). The diets were the same as fed to the parent (F₀) generation which had been mated at 12wks.

Groups of 10 males and 10 females of each experimental group were killed at week 24. Organ weights - liver, kidneys, heart spleen, brain and gonads and histology - liver, kidney, brain, gonads and small intestine were examined and recorded. Haematological parameters were examined - Hb, Hct, WBC; as was clinical chemistry - serum glutamic-pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT). All survivors were killed at week 36 and investigated for the same parameters.

The diet was well tolerated, i.e. no palatability problems occurred. Males fed the lupin diet showed a greater overall gain in body weight than the control group. No differences were observed in females. Food consumption and food conversion efficiency were similar in both dietary groups.

The relative liver weights of the treated rats (M and F) were significantly lower than those in the control group. There were no treatment related haematological effects at any stage in either generation. No gross pathological changes were observed at autopsy, and all histopathology was normal in both generations.

Summary: The only evidence of toxicity was a reduction in relative liver weight at 12 mg/kg bw/day.

In a study by Robbins et al (1996), groups of 20 male and 20 female Sprague-Dawley rats (3wk old) were fed diets containing lupin alkaloids of *L. angustifolius* at dose levels of 0, 100, 330, 1000 or 5000 ppm (equivalent to 0, 10, 33, 100 and 500 mg/kg bw/day) for 90 days. The alkaloid profile consisted of lupanine 42- 59%, 13-hydroxylupanine (24-45%, α -isolupanine (1-15%) and <1% total of other trace alkaloids.

There were no deaths among the rats at any of the dose levels and no changes in behaviour attributable to alkaloid ingestion. Parameters examined included: body weight; food intakes; haematology - White Blood Cell (WBC) count, Red Blood Cell (RBC) count, platelet (Plt), Mean Cell Volume (MCV), Haematocrit (Hct); organ weights - adrenals, brain, heart, kidney and liver; and histopathology - liver, heart and bone marrow.

The group mean body weights of male rats fed a diet containing 500 mg/kg bw/day lupin alkaloids were significantly lower than the control group from day 3 to the end of the study. The body weights of rats fed 100mg/kg bw/day were also lower, but only significantly so on a few occasions. This observation was similar for female rats. Food intakes were observed to be consistently slightly lower in both sexes given 100 and 500 mg/kg bw/day diets. All effects were reversible once normal diet was resumed.

There was no evidence for a treatment-related response in the haematological or clinical chemistry findings. The histopathological examination was unremarkable.

Organ weights showed no obvious consistent differences between treated and control groups. Kidney and heart weights showed some inconsistent differences between sexes that were not dose-related. Adrenal gland weights were unaffected by the treatment. Relative liver weights for both males and females at the 500 mg/kg bw/day dose level were significantly higher than control groups. Relative brain weights were

significantly higher than control groups in M and F 500 mg/kg bw/day groups. The changes could be ascribed to the lower overall body weights of these groups.

Summary: The only toxicological effect was reduced bodyweights and increased liver weight at 500 mg/kg bw/day. The NOEL was 100 mg/kg bw/day.

In a study by Butler et al (1996), three groups of 20 male and 20 female Sprague-Dawley rats were fed *ad libitum* with diets based on lupin (*Lupinus angustifolius*) flour to provide dietary alkaloid concentrations of 250, 1050 or 5050 ppm (equivalent to 25, 105, 505mg/kg bw/day) for 90-98 days. A control group was fed 50 ppm alkaloid (equivalent to 5 mg/kg bw/day) which was the background level in the flour used to formulate the diets. The alkaloid profile of the flour consisted of 42-59% lupanine, 24-45% 13-hydroxylupanine, 7-15% angustifoline and 1- 1.5% - isolupanine to reflect the alkaloid profile in surveyed commercial lupin cultivars (data provided by the Grain Pool Western Australia).

Examination parameters included body weight, food and water intake; haematological examination - total red blood cell count, white cell count, mean cell volume, haemoglobin (Hb), Haematocrit, differential and reticulocyte count; urine chemistry; gross histology and organ weight; histopathology.

No rats died in the study, or demonstrated any behavioural changes attributable to treatment with alkaloids. Slight transient weight loss was recorded in the male group treated with the lowest concentration (25 mg/kg bw/day) dose but this was not considered to be related to the treatment. Food intakes were generally equivalent with small sporadic variations between treatment and control groups. Water intakes were higher in all female groups from day 3-7 and for the duration of the study. This was not dose-related. Males showed no significant difference in this parameter.

A small statistically significant dose-related decrease in RBC and Hct was observed in male and female rats examined during days 42-49 for all dose groups. In males a significant non-dose related decrease in MCV was observed in all treatment groups. At the end of the study, a statistically significant decrease in Hct and MCV in males was observed in all treatment groups; the decrease in Hct showed dose-dependence, MCV did not. In females MCV values in low and high dose groups were increased. Hb was decreased in top-dose males and RBC was decreased in low dose females. Immediately prior to terminal kill, females treated at the highest dose showed an elevated WBC count. Differential and reticulocyte counts were normal.

Summary: Transient changes in Hct and MCV were not dose related and therefore could not be attributed to the treatment. The only treatment related effects at 505mg/kg bw/day were a decrease in Hb for males and increase in WBC for females. The NOEL was 105mg/kg bw/day.

Chronic Toxicity

In a study by Grant et al (1993, 1995) to examine the effect of feeding various various legumes to rats on body composition and organ weight, one group of 48 rats was fed a lupinseed-based diet (*Lupinus angustifolius*) for approximately 2 years. All the legume-based diets reduced feed conversion efficiency and growth rates during the initial 250

days. After 250 days, bodyweight gains were similar to controls. There was no significant change in body composition or organ weights as a result of consumption of lupin seed. Caecum and colon weights were significantly increased in treated rats although these effects did not appear to be mediated by the lectin or protease inhibitor content as these effects were not seen with other legumes containing high lectin and protease inhibitors. The effects are more likely to be the result of production of volatile fatty acids from digestion of dietary fibre.

While this study was not designed specifically to study the long-term effects of consumption of lupin alkaloids, there was no evidence of toxicity in rats from consumption of a lupinseed-based diet.

Developmental Toxicology

Anecdotal stories implicate lupin alkaloids as a teratogen of cattle following maternal ingestion of lupins containing the lupin alkaloid anagyryne during gestation. The effects are reported to be crooked calf disease (arthrogryposis) (Cheeke, 1989). Anagyryne is not likely to be present in major cultivars for human consumption and currently plant breeding programs are ensuring that this remains the case.

In a study by Panter et al, in Garland and Barr (1998), seven cross-bred 2-3yr old prima-parous heifers were bred to a Hereford bull and divided after confirmation of pregnancy into three groups to test the developmental effects of piperidine alkaloids in the diet. Group 1 were fed *L.formosus*, Group 2 were fed *L. arbustus*, and Group 3 were controls. Results are summarised at Table 3.

Table 3: Developmental and maternal effects of ingestions of lupin alkaloids

Group	Cow number	Lupin	Dose mg/kg bw/day	Effects in cows	Effects in calves
1	1	<i>L. formosus</i>	122	maternotoxic effects at 122 and 133;	One calf with cleft palate.
	2		133		
	3		84	no clinical signs.	
2	1	<i>L. arbustus</i>	42	no clinical signs;	One calf died in utero; the other suffered from apparent developmental delay.
	2		42	no clinical signs.	
3	?	Control	0	no effects	no effects

Each cow was dosed by gavage twice daily (AM and PM) through gestation days 40-49. *L. formosus* contained high levels of ammodendrine and N-acetyl hystrine (and no N-methyl ammodendrine), while *L. arbustus* contained ammodendrine and N-methyl ammodendrine (and no N-acetyl hystrine). These were given as dosages ranging from 84mg/kg bw/day to 133mg/kg bw/day alkaloids for Group 1, and 42mg/kg bw/day for Group 2.

Lupinus formosus caused clinical toxicoses in cows 1 and 2 (at the dosages of 122 and 133mg/kg bw/day) in group 1 by day five of the feeding study. No effects were observed at the dose of 84mg/kg bw/day for cow 3. Neurological symptoms of

intoxication in affected cows included depression, anorexia, frothing, muscular weakness and ataxia. Rapid recovery was observed when feeding of lupins ceased. No clinical signs were observed in Group 2 which were fed lower levels of alkaloids. One calf in Group 1 had full bilateral cleft palate, it is unclear at which dose level the mother was exposed, others were normal. In Group 2 one calf was born with contractures of carpal joints which resolved within three weeks, the other foetus died *in utero*.

This study is limited due to the small sample size and poor quality of reporting. While there is some evidence of teratogenicity from these studies, they are considered inadequate to assess the teratogenicity of lupin alkaloids.

Calves of normal healthy cows are occasionally born with contractures of the carpal joints that resolve early after birth, so this effect may not be due to alkaloids.

Reproductive Toxicity

In a modified reproductive study by Ballester et al (1984), two generations (F₁ and F₂) of groups of 20 male and 20 female weanling Wistar rats were fed diets based on sweet lupin flour from *L. albus*, containing 41.8% protein and 0.025% lupanine for nine months. Three comparable batches were prepared to give a diet containing 20% lupin protein, and alkaloid level of 0.012% alkaloid/diet (equivalent to 12mg/kg bw/day). The diets were the same as fed to the parent (F₀) generation which had been mated at 12wks. F₃ generation was bred and examined for effects but did not form part of the feeding study. The F₂ generation was obtained by mating at 12wk of age 5 male and ten females from the F₁ generation. Treatment diet continued during the mating period (over 3wks), lactation and weaning. Males continued as per the feeding study treatment group. Reproductive performance of parent and subsequent generations were investigated.

Records were made of the number of pups in each litter, and total weight of the litter on days 1, 6, 16 and 21. When weaned, 20 males and females of each dietary group were selected from as many F₂ litters as possible and maintained. An F₃ generation was obtained in the same manner as the previous ones. All F₁ and F₂ dams continued as part of the 9-month chronic study.

Fertility was similar in all generations. Growth rate of lupin fed F₂ pups was the same as their control group peers. F₃ pups did not show any treatment-related differences in growth from day 1 until weaning (21 days).

Results from a previous multigenerational feeding study, which was not available, (Tannous et al, cited in Ballester et al 1980), using diet based on 60-100% *L. luteus* seed were cited in the above study. The following effects were reported: mature rats grew and mated normally when fed an 80-100% lupin seed diet, however, litter deaths were noted after the 80% diet and; no live litters were produced by rats given diets containing only *L. luteus* seed. The results of this study have been cited to show that high or total levels of *L. luteus* seed in the diet have serious impact on the ability of rats to produce viable offspring - conception and pregnancy occur but either no live

litters are produced, or they die soon after birth. These effects could be attributed to nutritional impairment induced by total lupin diet rather than toxicity.

Summary- there were no observed adverse effects on fertility, lactation or any other reproductive parameters in rats at the dietary level of 12mg/kg bw/day lupin alkaloids.

Genotoxicity

There is no evidence that the lupin alkaloids are mutagenic (BIBRA, 1986).

Neurotoxicity

In a series of studies on cats and dogs, the ability of lupanine and sparteine to block transmissions through the ganglia were investigated. These lupin alkaloids were effective in antagonising or inhibiting electrically- and acetylcholine-induced hypotension and sino-carotid reflex. This is in addition to the neurological endpoints observed in developmental and acute toxicity studies. The lowest level where no effect was observed was 5mg/kg i.v in the dog and 0.5mg/kg i.v. in the cat. While it is difficult to extrapolate from an i.v. dose to dietary levels, the same neurological effects which were apparent in acute toxicity studies were reflected in this study.

Summary - Sparteine and lupanine appear to be active at a ganglionic level. Ganglionic transmission of the sympathetic nervous system is inhibited as evidenced by the inhibition of the sino-carotid reflex. In the parasympathetic nervous system, lupanine suppresses the effects of pre-ganglionic stimulation of the pneumo-gastric nerve.

Human Toxicity Studies

In humans, general toxicity symptoms attributed to ingestion of high levels of alkaloids are reported to include malaise, nausea, mydriasis, respiratory arrest, visual disturbances, ataxia, diaphoresis, progressive weakness, or coma. In the past, sparteine has been used as an oxytocic agent (to induce labour).

Most of the human studies are pre-1980 but are considered relevant to alkaloid toxicity. A literature summary (Keeler, undated, in Cheeke, 1989) of these papers prior to 1981 makes the following observations:

- Oral doses (acute) of bitter lupins showed that 11-46 mg/kg of mixed alkaloids was lethal in three of five cases, serious intoxication in the other two;
- Sparteine only proved lethal (acute dose) when ingested orally at >30 mg/kg;
- Sparteine sulphate (used as an oxytocic agent) induced serious toxicosis. Survival in one case at 55 mg/kg.

In the paper by Schmidlin-Maszaros (1973), seven cases of human poisoning were described, including four fatal cases. Three of fatal cases involved ingestion of lupin seed, probably *L. albus* containing about 2% alkaloids by children, and the fourth was the ingestion of 423 mg sparteine, in tablets, by a child. Two non-fatal poisonings of adults occurred after ingesting an estimated 2-3 mg alkaloids. Lowan et al (1995) reported a non-fatal poisoning of a woman in Australia who ate bitter lupin beans imported from Chile.

In relation to chronic toxicity in humans, there is some information available from the traditional consumption of debittered lupins in southern Europe as a snack food (Hondleman, 1986). These alkaloids are reported to contain approximately 500 mg/kg of alkaloids (Gross, pers. comm., cited in Petterson, 1985). If it is assumed that the intake of beans is approximately 50 g per day, the daily dose of alkaloids would be 0.35 mg/kg/day for a 70 kg adult. This dose level appears to be tolerated without any adverse effects, but may also be associated with some increased tolerance in these consumers. There is no data to indicate whether children can tolerate this level of intake.

Summary – On the basis of the case studies of human poisonings due to lupin alkaloids, the literature indicates that the acute lethal dose is approximately 30 mg/kg, which is considerably lower than for rodents and suggests that human are the most sensitive species for alkaloid toxicity. Traditional consumption of debittered lupins in Europe suggests a dose of 0.35mg/kg/day is without chronic effects for adults. There is no data in relation to potential adverse effects in children.

HAZARD CHARACTERISATION

Although the reports of the human studies are anecdotal or dated, they seem to indicate a marked difference in sensitivity between animals and humans with regard to acute toxicity. The reports of toxicity in humans, when taken together, indicate that the lethal dose is approximately 30 mg/kg, where the major alkaloid is sparteine. A 3-month study in rats, on the other hand, gave a NOEL of 90-105 mg/kg be/day, which suggests that the rat is not a suitable model for establishing levels of tolerable exposure in humans.

The only data available on human chronic toxicity are the reports of traditional use of lupini beans in Europe, which indicate a daily dose of 0.35 mg/kg can be tolerated in adults without adverse effects. On the basis of this limited data, however, it is not appropriate to regard this dose level as the safe level for all individuals in the population. The only data available on the levels of alkaloids in lupini beans is anecdotal – there seems to be no published information. Also, the information applies only to adults, not children, and it is likely that the adult population has developed a certain amount of tolerance to these alkaloids. The limited metabolism data available, however, suggests that the alkaloids are rapidly excreted unchanged, which would reduce the likelihood of chronic toxicity.

If a safety factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake (PTDI) for humans is 0.035 mg/kg/day or 35 µg/kg/day.

DIETARY EXPOSURE ASSESSMENT

There are no dietary survey data available from which to determine food consumption levels of lupin alkaloids since lupins currently have very limited use in foods. An estimate of potential exposure can be made if it is assumed that lupin flour could replace 10% of the wheat flour in any flour-based product. The 10% maximum level for lupin flour is justified based on technological reasons associated with the flour. Dietary exposure could therefore be estimated by assuming that of all flour products

in the market place, only 5% are likely to contain lupin flour at a level of 10% of total flour volume. Levels of dietary alkaloids can then be calculated based on the alkaloid concentration typically present in lupins harvested for human consumption - most recently reported being 130 mg alkaloids/kg seed.

Using this assumption and the mean, median and 95%ile consumption rates of wheat flour for all ages, obtained from the National Nutrition Survey (NNS), dietary exposure per day is calculated by assuming that average adult weight is 70 kg.

Table 4: Estimated lupin alkaloid exposures for all ages 2 years+

Wheat flour consumption (NNS 1995)	Flour likely to contain lupin flour (5% total)	Actual amount of lupin flour if 10% total flour	Alkaloid consumption if 130mg/kg in lupins	Daily dietary exposure
Mean - 105.8g/d	5.29g/d	0.529g/d	72.67µg/d	0.0010mg/kg bw/d
Median - 90.7g/d	4.535g/d	0.4535g/d	58.95µg/d	0.0008mg/kg bw/d
95% - 244.8g/d	12.24g/d	1.224g/d	159.12µg/d	0.002mg/kg bw/d

The data in Table 4 indicate that consumers of flour based products such as pasta, pastry and cakes and biscuits would be likely to have a daily exposure to lupin alkaloids of 2 µg/kg bw/day at the 95%ile of consumption. This is well below the estimated PTDI of 35 µg/kg bw/day. Normal consumers at the mean and median of consumption are well below this figure.

The level of exposure to alkaloids from home use of lupin seeds is difficult to assess. The European experience suggests that lupin seeds in the home are most likely to be consumed as a snack food. Low alkaloid varieties of lupin seeds contain approximately one quarter of the alkaloid content of de-bittered European lupini beans and thus are unlikely to cause symptoms of toxicity for the majority of the population. However, given the paucity of data, it is not possible to state that ingestion of these lupins will be without adverse effects for all individuals in the population.

There is little information available on the effect of heating or cooking on the stability of lupin alkaloids although they are known to be soluble in water as shown by debittering processes. Fudiyansyah et al (1995) showed a decline in the alkaloid content with cooking of *L. angustifolius* seed kernels from 70 to 20 mg/kg but this may have been due to leaching of the alkaloids into the cooking water. Lupin alkaloids are generally thought to be very chemically stable.

RISK CHARACTERISATION

The available data on lupin alkaloids is limited and does not allow a full characterisation of the risk of exposure to humans. It is of particular concern that the available data indicates that humans are more susceptible to the toxicity associated with lupin alkaloids than other species. The traditional use of debittered lupini beans in Europe as a snack food has been reported to be without adverse effects at a dose level of 0.35 mg/kg/day in adults. On the basis on this data, a tolerable level of exposure for humans has tentatively been established at 35 µg/kg/day using an

uncertainty factor of 10 in order to account for the variable sensitivity in the human population.

The major potential source of exposure to lupin alkaloids is the use of lupin flour from low alkaloid varieties of lupins to substitute for a small percentage of wheat flour. The available information on potential exposure via lupin flour suggests that at current levels of use, human exposure will be well below this tolerable level of exposure.

There is also potential for exposure to these alkaloids through the use of lupin seeds as a snack food. At present, this practice is uncommon and confined to a sub-population of southern European immigrants. Given the uncertainty regarding the toxicity of lupin alkaloids, there may be cause for concern if this practice were to become commonplace, particularly if lupini beans (the large seeded bitter cultivars of *L. albus*) were to be widely marketed. The consumption of the lupini bean in Australia and New Zealand is currently not a concern since the current consumers understand the de-bittering process required. While the low alkaloid varieties grown in Western Australia have approximately one quarter the alkaloid content of debittered lupini beans, on the basis of the data available, it is not possible to state that ingestion of the low alkaloid varieties will be without adverse effects for all individuals in the population.

Further work

In order to characterise further the potential human risk associated with lupin alkaloids, additional research is required to establish the basis for the observed toxicity in humans.

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