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Alkaloids in edible lupin seeds

A toxicological review and recommendations

Kirsten Pilegaard and Jørn Gry

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Preface

The present report was prepared by the Nordic Project Group on Risk Assessment of Inherent Natural Toxicants in Food Plants and Mushrooms.

The Project group referred to the Nordic Working Group on Food Toxicology and Risk Evaluation (NNT) within Nordic Council of Ministers.

Assessment of health risks connected with exposure to naturally occurring toxicants in foodstuffs has become an important area for NNT in the recent years. A series of Nordic reports based on the work performed by the Nordic Project Group has been published:

Gry, J. and Pilegaard, K. (1991) Hydrazines in the Cultivated Mushroom (*Agaricus bisporus*). Vår Föda 43;Supplement 1.

Uggla, A. and Busk, L. (1992) Ethyl carbamate (urethane) in alcoholic beverages and foodstuffs – a Nordic view. Nordiske Seminar- og Arbejdsrapporter 1992:570.

Størmer, F.C., Reistad, R. and Alexander, J. (1993) Adverse health effects of glycyrrhizic acid in licorice. A risk assessment. Nordiske Seminar- og Arbejdsrapporter 1993:526.

Andersson, H.C., Slanina, P. and Koponen, A. (1995) Hydrazones in the false morel. TemaNord 1995:561.

Søborg, I., Andersson, H.C. and Gry, J. (1996) Furocoumarins in plant food – exposure, biological properties, risk assessment and recommendations. TemaNord 1996:600.

Gry, J. and Andersson, H.C. (1998) Nordic seminar on phenylhydrazines in the Cultivated Mushroom (*Agaricus bisporus*). TemaNord 1998:539.

Andersson, H.C. (1999) Glycoalkaloids in tomatoes, eggplants, pepper and two Solanum species growing wild in the Nordic countries. TemaNord 1999:599.

Andersson, H.C. (2002) Calystegine alkaloids in Solanaceous food plants. TemaNord 2002:513.

Andersson, H.C., Wennström, P. and Gry, J. (2003) Nicotine in Solanaceous food plants. TemaNord 2003:531.

Andersson, H.C. and Gry, J. (2004) Phenylhydrazines in the cultivated mushroom (*Agaricus bisporus*) – occurrence, biological properties, risk assessment and recommendations. TemaNord 2004:558.

Gry, J., Søborg, I. and Andersson, H.C. (2006) Cucurbitacins in plant food. TemaNord 2006: 556.

Beckman Sundh, U., Rosén, J. and Andersson, H.C. (2007) Analysis, occurrence and toxicity of ß-methylaminoalanine (BMAA). TemaNord 2007:561.

Andersson, H.C., Kristinsson, J. and Gry, J. (2008) Occurrence and use of hallucinogenic mushrooms containing psilocybin alkaloids. TemaNord 2008 (In press).

Lupin seeds have no history of use for human consumption in the Nordic countries. In Southern Europe seeds from white lupin (*Lupinus albus* L.) with high quinolizidine alkaloid content have been eaten as a snack food but only after most of the alkaloids has been removed. The use of seeds from unsuitable lupus species or cultivars or the use of unprocessed seeds may cause acute intoxication in humans. Low alkaloid containing lupin seeds from white lupin and from narrow-leaved lupin (*Lupinus angustifolius* L.) have within the last decade been introduced in Europe.

Anagyrine, a quinolizidine alkaloid structurally related to the quinolizidine alkaloids in the white and narrow-leaved lupins, from wild lupin species can cause developmental effects in ruminants. However, this quinolizidine alkaloid is not found in seeds from white and narrow-leaved lupin. At present Australia is the major producer of seeds from narrow-leaved lupin with low alkaloid content. White and narrow-leaved lupin are, however, already grown in Europe. Lupin flour, that is high in protein, can partly replace wheat flour in bread, cakes and pasta. Seeds from the narrow-leaved lupin with low alkaloid content, grown in Australia, underwent a risk assessment as a novel food in 1996 in the United Kingdom. Here a level of total quinolizidine alkaloids in seeds, similar to the level already legally operative in Australia, was recommended. The French authorities have regulated the content of quinolizidine alkaloids in a low alkaloid cultivar of white lupin. Lupin seeds are also found in foods marketed in the Nordic countries but neither the species/cultivars nor the contents of quinolizidine alkaloids have been regulated in the Nordic countries. The present report aims at reviewing and summarizing the chemical and toxicological data on quinolizidine alkaloids in seeds of *L. albus* and *L. angustifolius* and at estimating the potential risk from the dietary exposure to quinolizidine alkaloids from these lupin species.

Literature about quinolizidine alkaloids in white lupin (*L. albus* L.) and narrow-leaved lupin

(*L. angustifolius* L.) was identified by searches up to 4 January 2008 in BIOSIS previews 1969–2006, Food Science and Techonology Abstract retrospective 1969–1989 and 1990–2008/01, MEDLINE 1966–(June 2004/06) and MEDLINE In Process & Other Citations Jun Wk 3 (2004/06) and PubMed December 2007. The search terms were lupinus albus, lupinus angustifolius and the combined search terms toxic, lupin and alkaloid. Literature on lupins used as fish fodder was excluded. The reference lists of identified publications were screened for additional references not collected in the data search.

The *Project Group* consisted of the following members:

- *Jørn Gry (coordinator)*, National Food Institute, Technical University of Denmark, Denmark
- *Christer Andersson,* National Food Administration, Sweden
- *Jan Alexander,* National Institute of Public Health, Norway
- *Anja Hallikainen,* National Food Agency, Finland
- *Arne Vidnes,* Norwegian Food Safety Authority, Norway

The present report has been prepared by Kirsten Pilegaard[1] and Jørn Gry[1] and accepted after thorough discussions in the Project Group and adopted by NNT in January 2008.

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Summary

Lupin seeds contain several bioactive substances with potential toxic effects in humans, especially quinolizidin alkaloids. The present report reviews the occurrence and toxicity of these alkaloids and estimates the risks from consumption of foods containing lupin seeds in the Nordic countries.

Lupin seeds have not until more recently been part of the human diet in the Nordic countries, but are now increasingly used, e.g. in bread and pasta, partially substituting wheat flour, and as snacks.

All lupin species studied contain quinolizidin alkaloids. Up to 500 lupin species occur worldwide, but only 12 in the Old World, and only seeds from few species have been used for human consumption ("edible lupins"), especially white lupin (*Lupinus albus* L.) and narrow-leaved lupin (*Lupinus angustifolius* L.).

Lupin varieties are often referred to as "bitter" when the total content of alkaloids is higher or equal to 10,000 mg/kg dry seeds and "sweet" when the content is lower or equal to 500 mg/kg dry seeds.

Traditionally, seeds from the edible lupin species contain high concentrations of bitter tasting and toxic quinolizidine alkaloids. Therefore, a debittering process, including cooking followed by soaking in water and daily replacement of water until bitterness disappears has been necessary before the seeds could be safely consumed. However, lupin seeds from cultivars low in alkaloids have been introduced in Europe for human consumption within the last decade.

In order to ensure safe use of lupin seeds in foods, the Advisory Committee on Novel Food and Processes in UK (ACNFP) concluded in 1996 that seeds from the low alkaloid lupin, the narrow-leaved lupin, are safe to use in the production of foods for human consumption provided that the level of lupin alkaloids in the seeds or derived lupin products does not exceed 200 mg/kg (and that the level of the mycotoxins phomopsins does not exceed 5 µg/kg). These recommended maximum levels for alkaloids and phomopsins were the same as the legal limits already operative in Australia. In 1998 France accepted the use of up to 10% of lupin flour made from a low alkaloid containing variety of white lupin called ARES as a food ingredient provided that the alkaloid content did not exceed 200 mg/kg.

Humans, especially children, are apparently much more sensitive to acute toxic effect from the alkaloids occurring in the "edible lupins" (the white and in the narrow-leaved lupins). Oral LD_{50}-values for the alkaloids in rats range from 1700-2300 mg/kg bw. In comparison severe acute in-

toxications in humans have been reported at estimated intakes, which are two orders of magnitude lower.

Subacute/subchronic and feeding studies in animals have mainly shown reduced body weight, often with concomitant reduced food intake, but the studies are considered to be only of limited value for prediction of possible toxicity in humans caused by exposure to lupin seeds.

With respect to reproductive and developmental toxicity, there are only few studies available on the "edible lupin" seeds. There are some indication of effects, but the results are questionable due to the design of the studies. However, grazing on non-edible lupins (*Lupinus taxiflorus, L. caudatus* and *L. nootkatensis*) have been connected with developmental effect in domestic animals, where the teratogen is belived to be the quinolizidin alkaloid anagyrine. It is noted that the North American lupin *L. nootkatensis* has been introduced in Iceland and now is widely spread in the country. Another lupin species, *Lupinus consentinii* has caused numerous cases of developmental effects in lambs. The suspected teratogen is multiflorine, which is structurally related to anagyrine.

It is not either clear whether the occasionally high amount of multiflorine in white lupins may be of concern.

Further, the apparently high sensitivity to acute intoxication, especially in children should be better studied.

Exposure to lupin alkaloids in the Nordic countries has been estimated based on highest recommended, as well as highest but still relevant levels of alkaloids, maximum use of lupin seeds in bread, pasta and snacks and high intakes of these three food categories. The estimated exposures are accordingly for children (weighing 20 kg) 0.6 – 1.4 mg/kg b.w. and for adults (weighing 60 kg) 0.3 – 0.8 mg/kg b.w.

For comparison case reports indicate that acute intoxications of adults caused by lupin alkaloids from "edible lupins" may occur after intake of 25-46 mg lupin alkaloids per body weight and case stories concerning small children indicate that intake of 11-25 mg/kg b.w. may be lethal.

In order to better ensure the safe use of lupin seeds in Nordic foods a series of recommendations are given concerning selection of proper lupin seeds for food use, analysis/exposure and toxicity data.

Sammendrag

Lupinfrø indeholder adskillige bioaktive stoffer med muligt toksiske virkninger i mennesker, især quinolizidinalkaloider. Rapporten resumerer forekomst og toksicitet af disse alkaloider og vurderer risikoen ved at indtage fødevarer, der indeholder lupinfrø i Norden.

Det er først for nyligt, at lupinfrø er blevet en del af den nordiske kost, men de bruges nu i stigende omfang, fx som snacks samt i brød og pasta, hvor de delvist kan erstatte hvedemel.

Alle de lupinarter, som er undersøgt, indeholder quinolizidinalkaloider. Der forekommer op imod 500 lupinarter i verden, heraf dog kun 12 i Europa. Kun få arter anvendes i fødevarer ("spiselige lupiner"), specielt Hvid Lupin ((*Lupinus albus* L.) og Smalbladet Lupin (*Lupinus angustifolius* L.).

Lupiner kaldes ofte "bitre", når det to tale indehold af alkaloider i frøene er større end eller lig med 10.000 mg/kg tørvægt, og "søde" når indholdet er mindre end eller lig med 500 mg/kg tørvægt.

Frø fra de traditionelle spiselige lupinarter indeholder høje koncentrationer af bittert smagende og toksiske quinolizidinalkaloider. Det har derfor været nødvendigt med en afbitringsproces, som omfatter kogning efterfulgt af iblødsætning i vand samt daglig erstatning af vand indtil den bitre smag er forsvundet, før frøene har været sikre at indtage. Imidlertid er der i det sidste årti indført lupinfrø til konsum fra sorter med lavt indhold af alkaloider til Europa.

I Storbritannien har the Advisory Committee on Novel Food and Processes (ACNFP) i 1996 konkluderet, at frø fra den Smalbladede Lupin med lavt alkaloidindhold, er sikre at anvende til fremstilling af fødevarer forudsat at indholdet af lupinalkaloid i frøene og lupinprodukter ikke overstiger 200 mg/kg (og at indholdet af mykotoksinerne phomopsiner ikke overskrider 5 µg/kg). Disse anbefalede maksimum indhold for alkaloider og phomopsiner er de samme som de grænser, der fastsat i australsk lovgivning. Fra 1998 accepterer Frankrig anvendelsen af op til 10% lupinmel fremstillet af en lav sort af Hvid Lupin (ARES) med lavt indhold af alkaloider som fødevareingrediens, forudsat at alkaloidindholdet ikke overstiger 200 mg/kg.

Mennesker, især børn, er tilsyneladende langt mere følsomme overfor akutte toksiske virkninger fra alkaloiderne som forekommer i "spiselige lupiner" (Hvid Lupin og Smalbladet Lupin).

Orale LD_{50}-værdiet for alkaloider i rotter er af størrelsesordenen 1700-2300 mg/kg legemsvægt. Til sammenligning er der set er alvorlige akutte forgiftninger i mennesker ved indtagelser af alkaloider, i mængder som er skønnet at være 2 størrelsesordner mindre.

Subakutte/subkroniske og fodringsundersøgelser i dyr, har hovedsageligt vist formindsket kropsvægt, ofte ledsaget af reduceret foderindtagelse, men undersøgelserne vurderes kun at have begrænset værdi til forudsigelse af mulig toksicitet i mennesker efter indtagelse af lupinfrø.

Der foreligger kun få undersøgelser af "spiselige lupinfrø" og deres effekt vedrørende reproduktion og teratogenicitet. Der er nogen indikation af effekter, men der kan stilles spørgsmålstegn ved resultaterne på grund af forsøgenes design. Imidlertid har husdyrs græsning af ikke-spiselige lupiner (*Lupinus taxiflorus, L. caudatus* og *L. nootkatensis*) været sat i forbindelse med teratogene virkninger, hvor det teratogene stof antages at være quinolizidin alkaloidet anagyrin. Det skal bemærkes, at den nordamerikanske lupin *Lupinus nootkatensis* er blevet indført i Island og den findes nu vidt udbredt i landet. En anden lupinart, *Lupinus consentinii* har givet anledning til adskillige tilfælde af teratogene effekter hos lam. Stoffet multiflorin, som er strukturelt beslægtet med anagyrin, mistænkes for at være det teratogene stof.

Det er ikke klart, om de lejlighedsvise høje mængder af multiflorin i Hvid Lupin kan udgøre en sundhedsmæssig risiko for mennesker. Endvidere bør den tilsyneladende store følsomhed for akutte forgiftninger, især hos børn, undersøges bedre.

De beregnede indtagelser af lupinalkaloider i Norden er baseret på, dels den højest tilladte mængde af alkaloider, dels på den maksimalt mulige anvendelse af lupinfrø i snacks, pasta og brød og det højest anbefalede indtag af disse 3 fødevarer. På grundlag heraf er det højeste beregnede indtag hos børn (der vejer 20 kg) 0,6 – 1,4 mg/kg legemsvægt og for voksne (der vejer 60 kg) 0,3 – 0,8 mg/kg legemsvægt.

Dette kan sammenlignes med "case reports", der anfører akutte forgiftninger hos voksne efter indtagelse af 25-46 mg lupin alkaloider per kg legemsvægt og for små børn at indtag af 11-25 mg/kg legemsvægt kan være dødelig.

Med henblik på forbedret fødevaresikkerhed ved indtagelse af lupinfrø i Norden gives en række anbefalinger vedrørende valg af egnede lupinfrø til fødevarer, analyse/indtagelses- og toksicitetsdata.

1. Introduction

All lupin species studied contain quinolizidine alkaloids. Some of these alkaloids are bioactive food plant constituents with anticipated toxic effects in humans (for a definition see Gry et al., 2007). The exact number of lupin species within the genus *Lupinus* is unknown but a number between 150 and up to 500 species has been suggested (Aniszewski, 1993; Wink et al., 1995). Only seeds from few of these lupin species have been used for human consumption. The edible species include the white lupin (*Lupinus albus* L.) used in Southern Europe, the narrow-leaved lupin (*Lupinus angustifolius* L.) grown in Australia, and the pearl lupin (*Lupinus mutabilis* Sweet) grown in South America. It is not clear whether other lupin species e.g. yellow lupin (*Lupinus luteus* L.) have been or still are used in foods. At least the yellow lupin (as well as the white and the narrow leaved lupins) is included in the EU Novel Food Catalogue (EU, 2008)

Lupin seeds have not until very recently been part of the human diet in the Nordic countries but now constitute a minor part of the diet. Ground lupin seeds from white and narrow-leaved lupins, that is high in proteins and low in alkaloids, can be incorporated into fodder for pigs, beef cattle, dairy cows, sheep and chicken. In addition, ruminants can graze on lupin plants or stubble (Edwards and van Barneveld, 1998; Allen et al., 1983). Lupins in general are of agricultural interest as a green manure and as a crop that can fight or control soil erosion (Lopez-Bellido and Fuentes, 1986; Aniszewski, 1993). They can reduce the use of fertilizers because of their ability to fixate nitrogen from the air due to the symbiosis between lupins and nitrogen fixing bacteria on their roots (Aniszewski,1993). Six European countries harvested lupin seeds in 2005, the latest year for which data are estimated (FAO, 2008). The major producer country was Germany (7720 tons seeds), followed by Poland (4046 tons), France (715 tons), Italy (300 tons), Greece (40), and Hungary (25 tons). In comparison the Australin lupin seed production was estimated to 42392 tons in 2005 (FAO, 2008). No data on the lupin species are available. FAO does not give any production data for lupin seeds for the Nordic countries. However, in Denmark the area grown with predominantly narrow-leaved lupins increased from 0 to 4,000 hectares from 2000–2004 (B. Jørnsgaard, personal communication, 2004).

Traditionally, seeds from the edible lupin species contained high concentrations of bitter tasting and toxic quinolizidine alkaloids. Therefore, a debittering process, including cooking followed by soaking in water and daily replacement of water until bitterness disappears has been necessary before the seeds could be safely consumed (Joray et al., 2007). In southern Europe debittered *L. albus* seeds have been used for human consump-

tion as a snack food. White lupin seed was considered to be an important food plant in Europe in 1997 (Gry et al., 1998; Pilegaard et al., 2007).

The idea of breeding lupin cultivars with a low alkaloid content emerged already early in 1900 century in Germany (Gladstones et al., 1998). However, lupin seeds low in alkaloids were first introduced in Europe for human consumption within the last decade. Since seeds with low alkaloid content deriving from the narrow-leaved lupin (*L. angustifolius*) were introduced on the European market before 15 May 1997, they do not fall within the EU novel food regulation (1997/258/EC). The seeds had before the introduction in the United Kingdom been subject to an evalution by the national Advisory Committee on Novel Food and Processes (ACNFP, 1996). This committee concluded in 1996 that seeds from the low alkaloid lupin, the narrow-leaved lupin, are safe to use in the production of foods for human consumption provided that the level of lupin alkaloids in the seeds or derived lupin products do not exceed 200 mg/kg (and that the level of the mycotoxins phomopsins does not exceed 5 µg/kg). These recommended maximum levels for alkaloids and phomopsins were the same as the legal limits already operative in Australia (ACNFP, 1996). In 1998 France accepted the use of up to 10 % of lupin flour made from a low alkaloid containing variety of white lupin called ARES as a food ingredient provided that the alkaloid content did not exceed 200 mg/kg (Direction générale de la santé, 1998). In Australia strict limits are imposed on seed alkaloid concentration of new varieties so that the average concentration is lower than 200 mg/kg dry matter (Gremigni et al., 2001). No information on such regulation in other countries is available.

The term ‘sweet lupin’ has been used for lupins with low alkaloid content and the term ‘bitter lupin has been used for lupins with high alkaloid content. According to Gremigni et al. (2001) the seed alkaloid concentration in the ’bitter’ varieties is higher or equal to 10,000 mg/kg dry matter whereas the concentration in the ’sweet’ varieties is lower or equal to 500 mg/kg dry matter.

Petterson (1998) reviewed the contents of macronutrients and other constituents in lupins. Lupin seeds consist of an outer part, a seed coat (hull), and an inner part, cotyledon (splits, meats). The seed coats are thick in lupin seeds and comprise about 25 % of the seed weight in *L. angustifolius* and 15 % of the seed weight in *L. albus*. The protein content in lupin seeds is 400 g/kg in the kernels of white and narrow-leaved lupin and 361 and 322 g/kg in the hulls from *L. albus* and *L. angustifolius*, respectively. The protein values after commercial dehulling of the seeds is 35 % from the cotyledon and 7–10 % for the hulls. The seeds are deficient in the amino acids lysine and methionine when compared to FAO standards. The crude fat content is 91 g/kg and 58 g/kg in the hulls of *L. albus* and *L. angustifolius*, respectively. In the cotyledon the content is 114 g/kg in *L. albus* and 66 g/kg in *L. angustifolius.* The composition of

the oil from *L. albus* is similar to most other edible oils except that it like cruciferous plant oils contain erucic acid. *L. albus* seed oil contains 1.5–2.7 % erucic acid (Petterson, 1998). The content of erucic acid in seeds is not regulated within EU but for edible fats and oils the maximum permitted erucic acid content is 5 % (Council Directive 76/621/EEC). The crude fibre content is high in the seed coat: 149 g/kg in *L. angustifolius* and 103 g/kg in *L. albus*. The carbohydrate composition in the cotyledon differs from the hulls. The carbohydrates in the hulls are structural polysaccharides: cellulose, hemicelluloses and pectins whereas the main carbohydrates of the cotyledon is non-structural polysaccarides consisting of galactose, arabinose and uronic acid (Petterson 1998).

The introduction of 'sweet' lupins has widened the use of lupins from the traditional use of debittered seeds as a snack food. Lupin seed flour can substitute part of the wheat flour used for bread, cakes and pasta. The ground seeds can substitute soy beans as an ingredient in e.g. ground beef or sausages, soy milk, and various fermented Asian food e.g. tempe and miso (Fudiyansyah et al., 1995; Petterson, 1998; Papavergou et al., 1999).

Cases of acute intoxication of adults and intoxication and even death of children after ingestion of raw or incompletely processed lupins with high alkaloid content have been reported. In some cases seeds from bitter varieties of *L. albus* have been identified. In other cases the lupin species have not been specified. The toxic substances causing these poisonings are the quinolizidine alkaloids in the lupins. Estimations of doses of lupin alkaloids causing acute intoxications indicate that children are more sensitive than adults (Schmidlin-Mezaros, 1973). Based on the limited data available it is estimated that humans might be two orders of magnitude more sensitive to acute effect than animals studied

Calves whose mothers had grazed on various wild American lupin species during pregnancy were born with developmental defects (crooked calves disease). The quinolizidine alkaloid, anagyrine, has been identified as the teratogenic substance. In Australia, a developmental defect affecting lambs whose mothers grazed on plants or seeds of the sand plain lupin (*Lupinus consentinii* Guss.) during mating or gestation have been reported. Also dwarfism in calves have been attributed to *L. consentinii*. The responsible alkaloid has not been identified but it has been noted that the predominant quinolizidine alkaloid in the plant, multiflorine, is structurally related to the teratogenic anagyrine (Allen, 1998; Allen et al., 1983). No anagyrine has been identified in seeds from *L. albus* and *L. angustifolius*. It should be noted that the multiflorine content in seeds from white lupin varies from 0 % to as high as 18 % (Muzquiz et al., 1994; El-Shazly et al., 2001; Wink et al., 1995; Als and Gade, 1997).

The use of lupins as food may also pose safety problems with mycotoxins, allergy and high manganese content unrelated to the content of quinolizidine alkaloids in the seeds.

Infestations of the seed with the fungus *Diaporthe toxica* Williamson, Highet, Gams & Sikisitham param (previously known as *Phomopsis leptostromiformis* (Kühn) Bubak syn. *P. rossiana* (Sacc.) Sacc. & D. Sacc) can result in the production of mycotoxins called phomopsins. Phomopsins exert their toxic effect by binding to microtubular proteins thereby inhibiting the formation of microtubules, that are essential for intra cellular transport mechanisms and cell division. Lupinosis, a disease caused by phomopsins on lupin seeds or other plant parts including lupin stubbble, has occured primarily in sheep but also in cattle, goats, donkeys, horses and pigs. The main feature of lupinosis is severe liver damage which often results in death. Lupinosis may cause abortion late in pregnancy of sheep and cattle and death of embryos in sheep early in pregnancy (Allen, 1998). If the maximum level of phomopsins in lupin is not exceeding 5 µg kg seed, the intake of lupin seed, does not cause concern for human safety (Inger Thorup, personal communication 2000).

After the introduction of lupin flour on the European market, allergic reactions, some of them severe, have been documented (Moneret-Vautrin et al., 1999; Radcliffe, 2005). In the Nordic countries, allergic reactions to lupin have been described from Norway (Fæste et al., 2004; Løvik et al., 2004). Studies show a relatively high risk of cross-allergy to lupin in between 30–60 % of persons who are allergic to peanuts. Due to the risk of lupins causing adverse reactions in susceptible individuals, labelling of foodstuffs containing lupin has become mandatory for all EU member states from 23 December 2007 (EU Commission Directive 2006/142/EC).

Manganese is a trace element, essential for normal brain development (Food and Nutrition Board – Institute of Medicine, 2000). It is however also a neurotoxicant in the event of high manganese uptake. Recently, there has been focus on the risk of accumulation of manganese in the brains of infants (aged 0–6 months) receiving infant formula with high content of manganese compared to what is found in breast milk. The infant is at special risk because the neonatal brain-barrier is not fully developed compared to later in life where regulatory mechanisms maintain homeostasis (Dobson et al., 2004). Manganese concentration ranging from 1060–4640 µg/g dry matter was found in seeds from 49 white lupins grown in Australia. High alkaloid lines had approximately two-thirds of the manganese concentration of low alkaloid lines Accumulation of manganese seems to be specific for seeds of white lupin and has not been reported for the narrow-leaved lupin grown in the same areas under comparable conditions. The manganese concentration in seeds of two high and one low alkaloid lines of *L. angustifolius* varied from 142–243 µg/g dry matter (Oram et al., 1979).

Safety problems with other constituents than quinolizidine alkaloids will be briefly reviewed later (Section 4.3). Information of these effects may be of importance for interpretation of data from studies with lupins otherwise aimed at studying the effect of quinolizidine alkaloids.

The aim of the present report is primarily to review the toxicity and evaluate the safety of the inherent natural toxicants, the lupin alkaloids, in seeds from white lupin (*L. albus*) and narrow-leaved lupin (*L. angustifolius*) (In Chapters 3–9). In Chapter 8 recommendations for the use of lupin seeds are given.

2. Chemical characterization

All plants of the genus *Lupinus* contain alkaloids, especially quinolizidine alkaloids (e.g. sparteine) b ut they can also contain piperidine alkaloids (e.g. ammodendrine and N-acetylammodendrine) and simple indole alkaloids (e.g. gramine). Examples of these three types of alkaloids are shown in Figure 1.

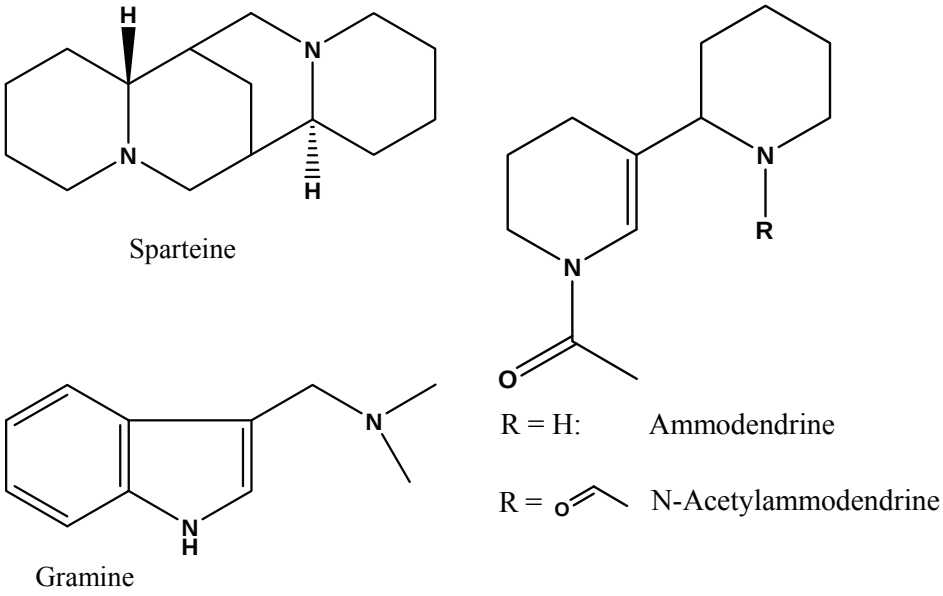
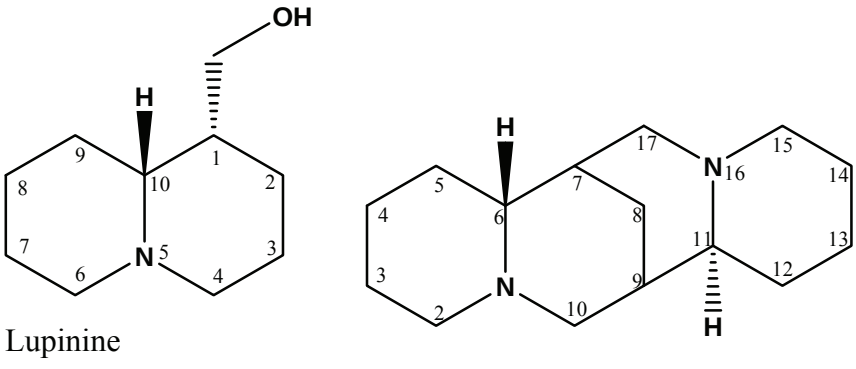


Figure 1. Structures of different alkaloid types in the genus Lupinus.

The alkaloids may occur i n all parts of the different lupin specie s. Totally, the hi ghest amounts are in th e seeds (see Chapter 4. Occurrence) . The alkaloids can be found as the free bases and as N-oxides and the hydroxylated alkaloids may also be esterified or glycosidated.

The most abundant and toxicologically important lupin alkaloi ds are the quinolizi dine alkaloids. They can be bicy clic (norlupi nane group), tricyclic (cytisane group) or tetracyclic (sparteine group). The numbering systems for t he bi- and tetracy clic quinolizine alkaloids are sh own in Figure 2.



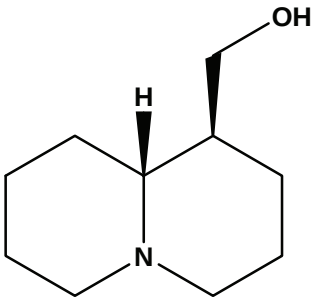
Lupinine

Sparteine

Figure 2. *Numbering system for bi- and tetracyclic quinolizidine alkaloids in the genus Lupinus.*

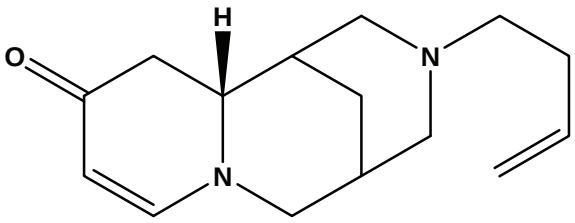
In Figure 3 is given the structures of quinolizidine alkaloids in a series of lupin species. The alkaloids are grouped into compounds derived from: 3a. norlupinane, 3b. cytisane and 3c sparteine.

3a. Norlupinane derivatives

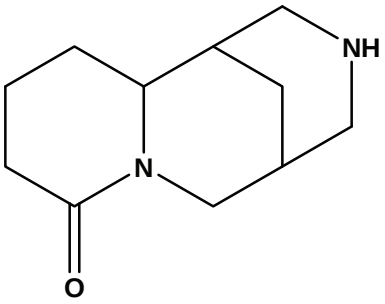


Epilupinine

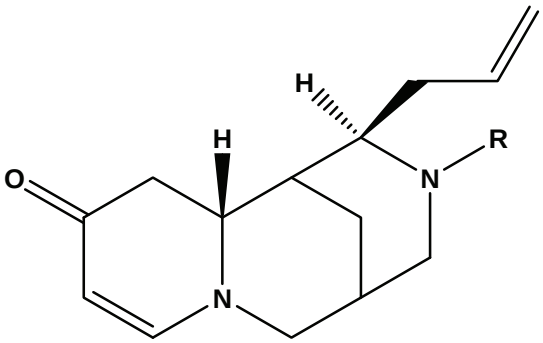
3b. Cytisane derivatives



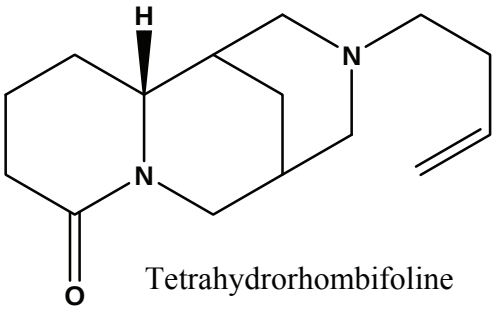
11,12-seco-12,13-Didehydromultiflorine



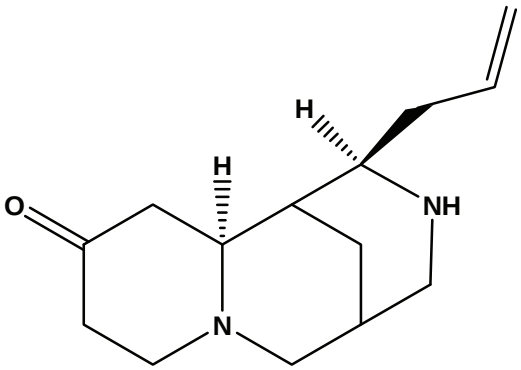
Tetrahydrocytisine



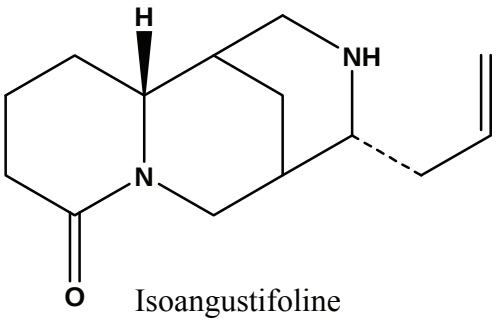
R = H: Albine
R = CHO: N-Formylalbine



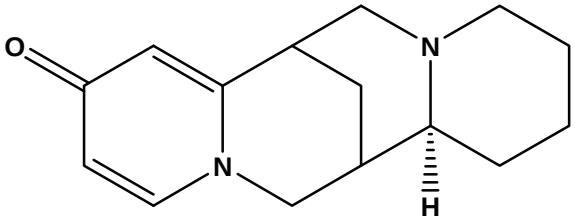
Tetrahydrorhombifoline



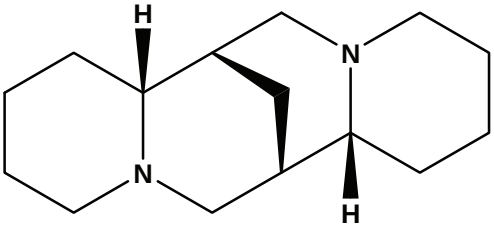
Dihydroalbine



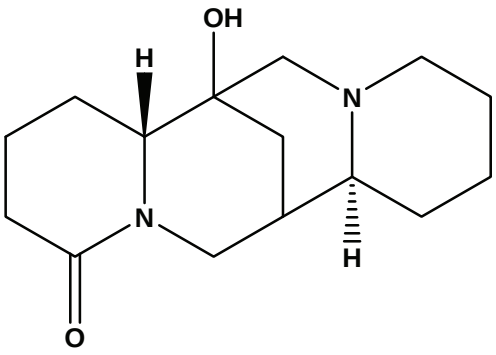
Isoangustifoline



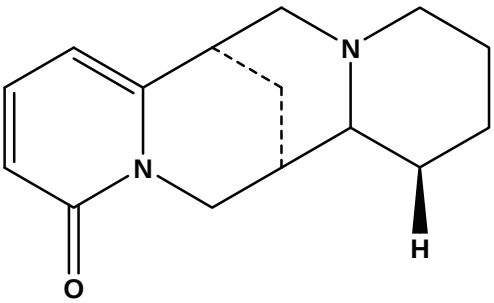
5,6-Dehydromultiflorine



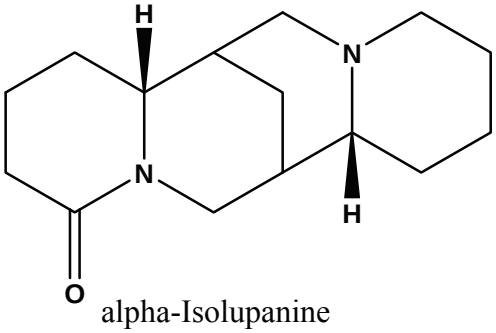
alpha-Isosparteine



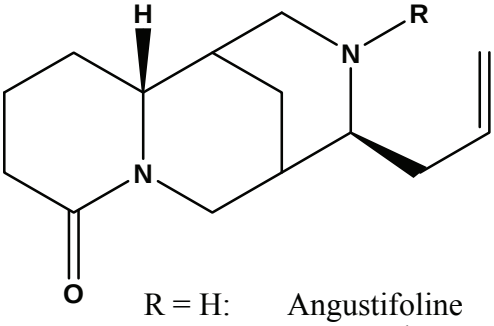
7-Hydroxylupanine



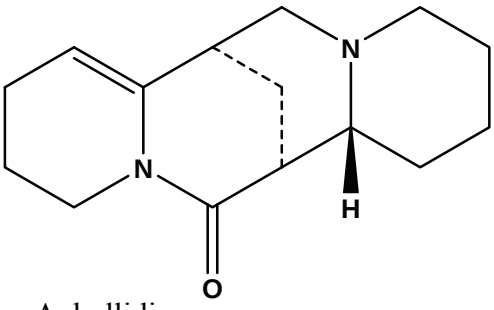
Anagyrine



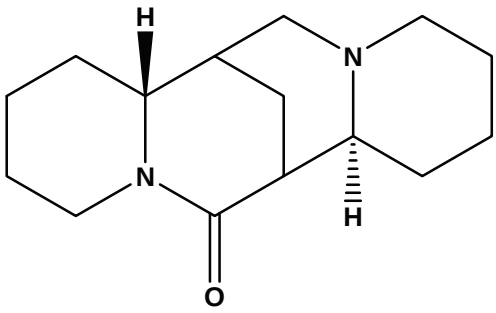
alpha-Isolupanine



R = H: Angustifoline
R = CHO: N-Formylangustifoline

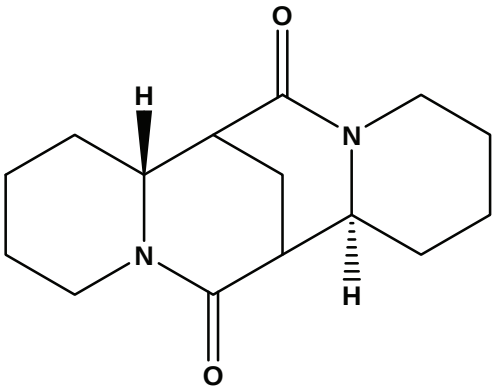


Aphyllidine

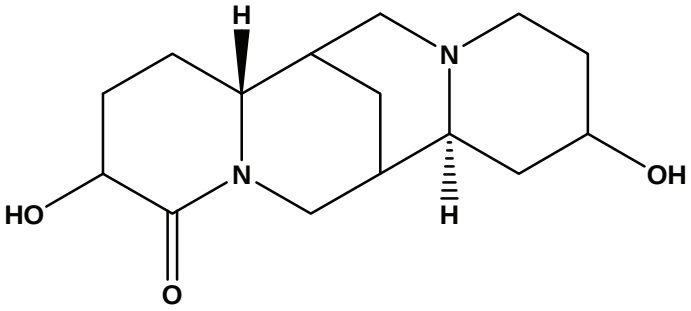


Aphylline

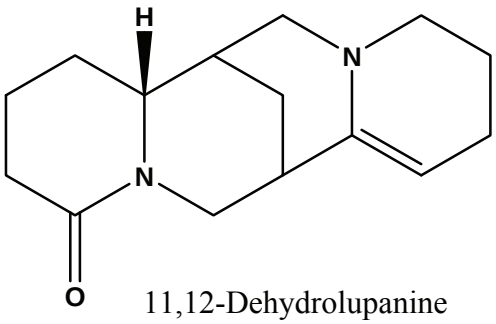
3c. Sparteine derivatives



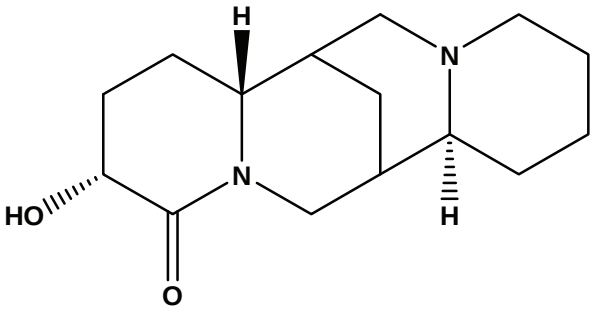
10,17-Dioxosparteine



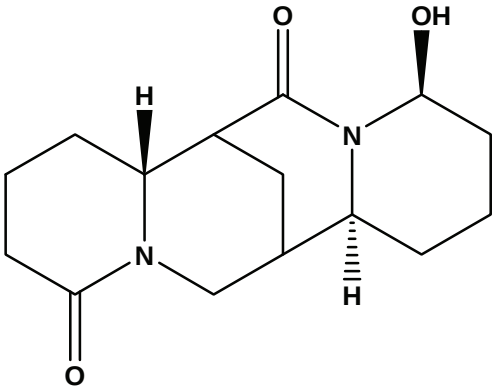
3,13-Dihydroxylupanine



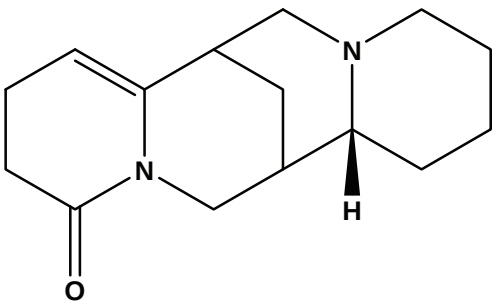
11,12-Dehydrolupanine



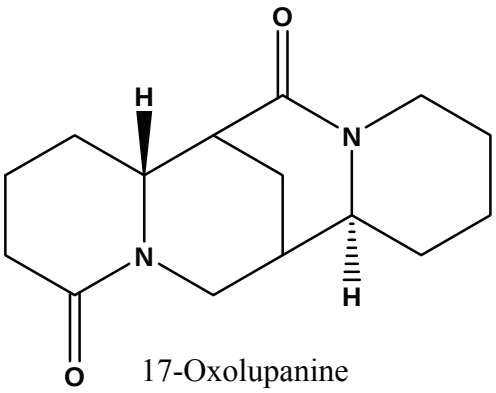
3beta-Hydroxylupanine



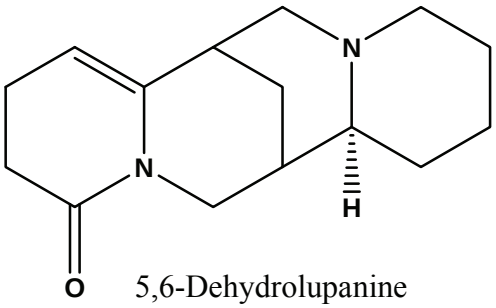
15beta-Hydroxy-17-oxolupanine



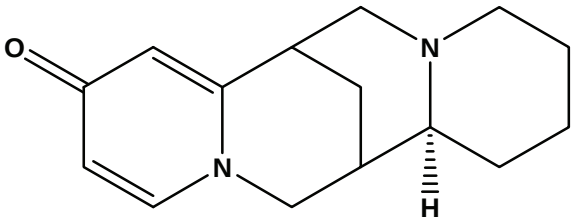
5,6-Dehydro-alpha-isolupanine



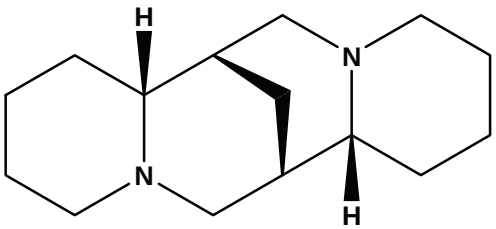
17-Oxolupanine



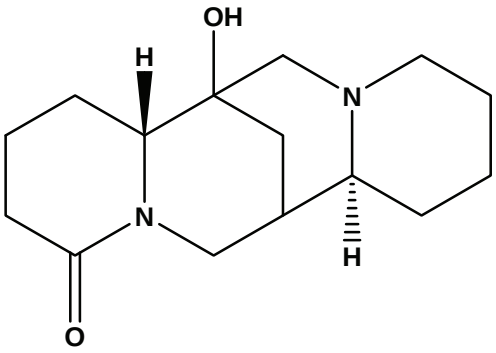
5,6-Dehydrolupanine



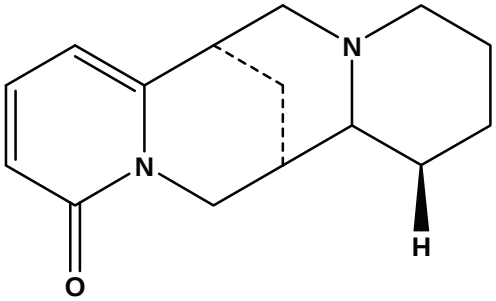
5,6-Dehydromultiflorine



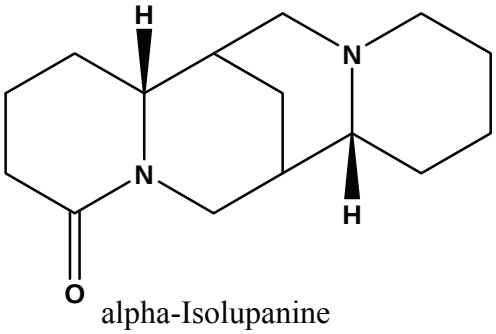
alpha-Isosparteine



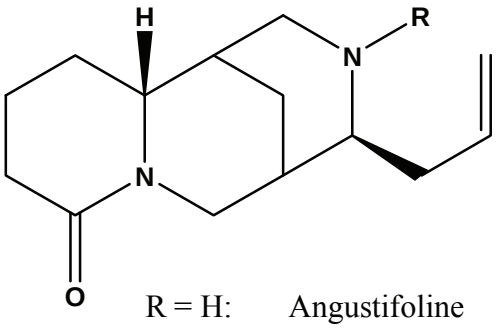
7-Hydroxylupanine



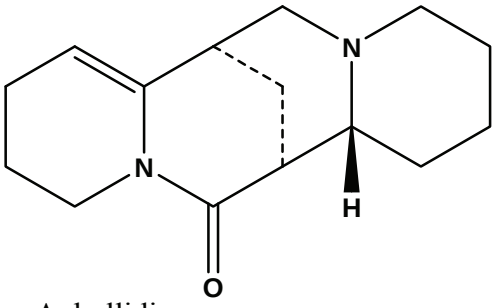
Anagyrine



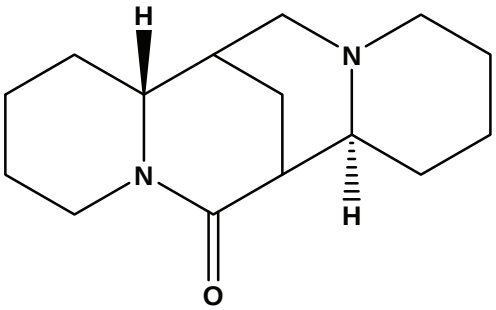
alpha-Isolupanine



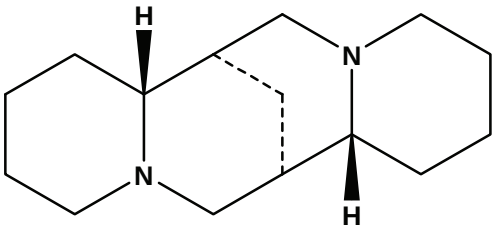
R = H: Angustifoline
R = CHO: N-Formylangustifoline



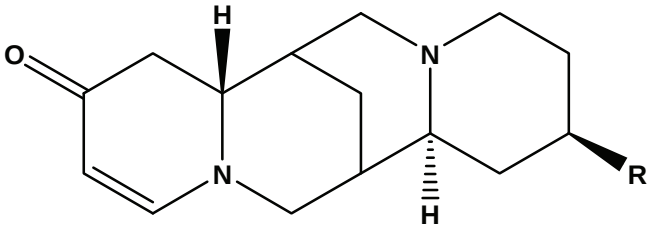
Aphyllidine



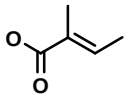
Aphylline

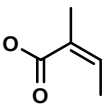


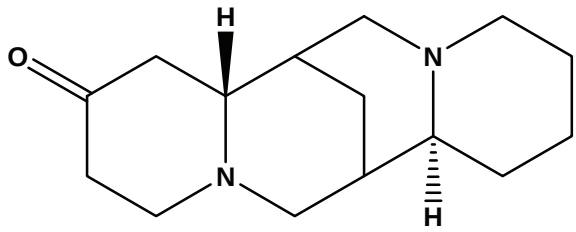
beta-Isosparteine



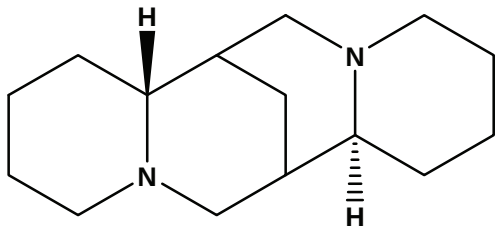
R = H: Multiflorine
R = OH: 13alpha-Hydroxymultiflorine

R =  13-Tigloyloxymultiflorine

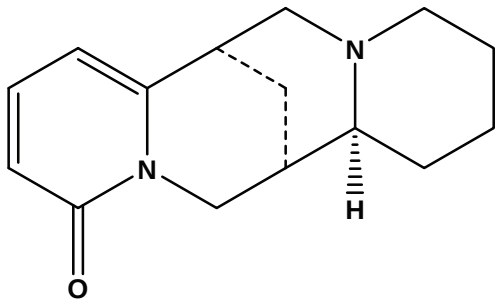
R =  13-Angeloyloxymultiflorine



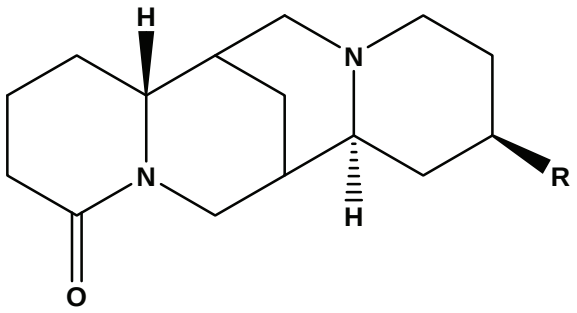
Dihydromultiflorine



Sparteine



Thermopsine

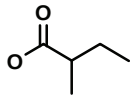


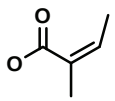
R = H: Lupanine

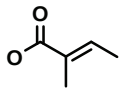
R = OH: 13-Hydroxylupanine

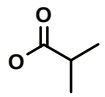
R =  13alpha-Acetyloxylupanine

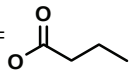
R =  13alpha-Propyloxylupanine

R =  13-(2-Methylbutanoyloxy)lupanine

R =  13alpha-Angeloyloxylupanine

R =  13alpha-Tigloyloxyupanine

R =  13-Isobutyryloxylupanine

R =  13-Butyryloxylupanine

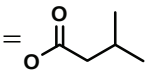
R =  13-Isovaleroyloxylupanine

Figure 3. *Quinolizidine alkaloids in different lupin species (Lupinus albus, L. angustifolius, L. caudatus, L. latifolius, L. formosus, L. argentus and L. consentinii). Three groups of alkaloids are shown: norlupinane - (3a), cytisane- (3b) and (3c) sparteine-derived groups.*

3. Occurrence

3.1 Lupin species

3.1.1. Edible lupin species

The exact number of lupin species has not been defined (Aniszewski, 1993). The taxonomy of the lupin genus (*Lupinus)* has given rise to much confusion and for instance some American species are taxonomically ill-defined. The Old World has 12 recognized lupin species (Gladstones,1998). Aniszewski referes in a review that there in the New World alone are 150 or other 200 species. Lopez-Bellido and Fuentes (1986) indicate in their review that the number of lupin species is between 300 and 400. According to Wink et al. (1995) the lupin genus is comprised of more than 500 species, whereas the EU Commission (2006) mentions 450 species.

Only few lupin species have been cultivated and used for human consumption. Apart from one species, the pearl lupin, originating from South America, all other edible species have their origin in Africa and the Mediterranean region. The four edible lupin species are briefly introduced below:

- White lupin or Egyptian lupin (*Lupinus albus* L. = *L. termis* Forssk.) (Zander, 1984; Hanelt, 2002). Hanelt (2002) classifies *L. albus* L. into one wild subspecies *L. albus* L. spp. *graecus* and a cultivated subspecies *L. albus* ssp. *albus*. The seeds are used in the Mediterranean area, North Africa and Australia. Both high and low alkaloid containing cultivars exist.
- The narrow-leaved lupin or European blue lupin (*Lupinus angustifolius* L = *L. varius* L.)(Zander, 1984, Hanelt, 2002). This species is extensively cultivated in Australia from the 1980s (reviewed by Aniszewski, 1993). Both high and low alkaloid containing cultivars exist.
- Yellow lupin (*Lupinus luteus* L.) has been used as green manure and fodder (Meyer, 1941, List and Hörhammer, 1976, Hanelt 2002). Due to the high content of alkaloids, yellow lupin is not normally used as a fodder without a debittering proces (List and Hörhammer 1976). To what extent it has been used for human consumption is unresolved, but according to List and Hörhammer (1976) the seeds have been used as a coffee substitute. Cultivars with high and low alkaloid content exist (Wasilewko and Buraczewska 1999).

- Pearl lupin (*Lupinus mutabilis* Sweet). An important food in the Andes region in South America. High alkaloid containing cultivars are cultivated (Gladstones 1998).

3.1.2 Non-edible lupin species

None-edible lupin species that have gi ven rise to p oisoning of a nimals and man are:

- Washington lupin (*Lupinus polyphyllus* Lindl.). Cultivated as a perennial in gardens throughout the Nordic countries. Acute poisoning of children after eating unripe seeds, that may be mistaken for peas or beans, or ripe seeds, that may be attractive to children because of their silky hairs, has been described but occur relatively seldom (Cooper and Johnson 1998, Frohne and Pfänder 1997). In Finland experiments with an alkaloid-poor variety has been carried out. The total alkaloid content in seeds from this cultivar varied from 226–366 mg/kg dry matter). Both the use of this lupin as green manure and the possibility of using the seeds for food or feed directly or after processing is mentioned (Aniszewski 1993).
- *Lupinus laxiflorus* Lindl., *Lupinus caudatus* and *Lupinus nootkatensis* Donn ex Sims are among the native *Lupinus* species in USA and Canada that when eaten by pregnant cows cause the congenital defect 'crooked calf disease' characterized by arthrogryposis, spinal curvature and cleft palate of calves. The quinolizidine alkaloid, anagyrine, is believed to be the teratogenic component in these lupins (Panter and Keeler 1993). It should be noted, that *L. nootkatensis* is the lupin widely grown in Iceland as an ornamental but not at least to obtain soil fertilization and prevent erosion. It has spread and settled in different regions of Iceland where it can be found on dry, poor, often disturbed soils. It also grows commonly along roadsides.
- *Lupinus latifolius* J.Agardh containing high amount of anagyrine has been suspected as a human teratogen. Bone deformities were observed in a baby whose mother had drunk milk from a goat foraging on the lupin. Anagyrine was detected in the milk. The goat gave birth to stillborn kids or kids with deformed legs. A dog fed the goat's milk delivered deformed puppies (Kilgore et al., 1981). No anagyrine was found in neither leaves nor seeds when this species was analysed for anagyrine much later (Wink et al., 1995).
- *Lupinus formosus* Greene and *Lupinus arbustus* Lindl. are teratogenic in cows. The high concentrations of the piperidine alkaloid, ammodendrine, in both lupin species, are thought to be responsible for the effect but other piperidine alkaloids (N-acetylhystrine and N-methylammodendrine) found in *L. formosus* may also play a role (Panter and Keeler, 1993; Panter et al., 1998). However, no trace of

ammodendrin was found in another sample of leaves and seeds of *L. formosus* but the leaves contained 75 % of N-acetylammodendrine (Wink et al., 1995).

- Silvery lupine (*Lupinus argentus* Pursh), which contains high levels of ammodendrine and N-methylammodendrine, gave rise to acute death in grazing cattle (Panter et al., 2001).
- The sand plain lupin or Western Australian blue lupin (*Lupinus consentinii* Guss.) has according to Gladstones (1998) incorrectly been called *L. digitatus* or *L. varius* in Australian literature before 1970. Numerous cases of hemimelia (incomplete development of the distal part of the limbs) in lambs whose mothers grazed on plants or seeds of this species during mating or gestation have been reported. The responsible alkaloid has not been traced but it has been noted that the predominant quinolizidine alkaloid in the plant, multiflorine, is structurally related to anagyrine (Allen, 1998; Allen et al., 1983). Dwarfism was reported in 14 % (20/145) of calves born to cows that had grazed on *L. consentinii* (Hawkins, 1994 cited of Allen, 1998).

3.2 Alkaloids in lupin species

3.2.1. Lupinus albus (white lupin)

The total alkaloid content in seeds of high and low alkaloid containing varieties of white lupin grown in various countries is shown in Table 1. Since there is not a general classification of whether the cultivars contain high or low levels of alkaloids or can be denoted as 'bitter' or 'sweet', the classification in bitter or sweet is based on the authors own description of the cultivars studied.

Table1. Total alkaloid content in seeds from white lupin (*Lupinus albus*) grown in various countries.

Cultivar	Grown in	No. of cultivars studied	Total alkaloid content (mg/kg dry weight)	Analysis	Reference
'Bitter'	Poland, Portugal	1	29,600	GC-MS	Aniszewski et al. (2001)
'Bitter'	Turkey, Greece, Italy, Sudan, the Azores, Spain, Portugal, Morocco, Egypt, Ethiopia	29	1,000–26,900	GC-MS	Múzquiz et al. (1994)
'Bitter'	Spain	1	30,700	GS-MS	de la Cudra et al. (1994)
'Bitter'	Egypt	40	2,000–14,000	Titrimetric	Christiansen et al. (1999)
'Bitter'	Egypt	1	15,600	MS	El-Shazly et al. (2001)
'Bitter'	Hungary	2	22,000–	PC	Haraszti and Vetter

Cultivar	Grown in	No. of cultivars studied	Total alkaloid content (mg/kg dry weight)	Analysis	Reference
			32,000		(1983)
'Bitter'	Denmark	2	320–470	GC-MS	Als and Gade (1997)
n.s.	Poland	3	600–950	TLC	Wasilewko and Buraczewska (1999)
n.s.	Poland	2	980–8,050	Photometry	Niwinska (2001)
n.s.	Poland	2	370–400	Photometry	Sujak et al. (2006)
'Sweet'	Poland, Portugal	1	580	GC-MS	Aniszewski et al. (2001)
'Sweet'	Turkey, Greece, Italy, Sudan, the Azores, Spain, Portugal, Morocco, Egypt, Ethiopia	20	100–4,200	MS	Múzquiz et al. (1994)
'Sweet'	Hungary	2	2,000–3,000	PC	Haraszti and Vetter (1983)
'Sweet'	Australia	n.s.	< 100	n.s.	Petterson and Mackintosh (1994)[1]
'Sweet'	Denmark	2	248–420	GC-MS	Als and Gade (1997)

[1] Data from an Australian national grain legumes database, GRAILE
GC: Gas chromatography
MS: Mass spectrometry
PC: Ascendent paper chromatography
TLC: Thin layer chromatography
Abbreviation: not specified: n.s.

An overview of the alkal oid profiles of various cultivars of white lupin *(Lupinus* albus) are shown in Table 2.

Table 2. Distribution of alkaloids (percentage) in seeds from white lupin (*Lupinus albus* L.).

Alkaloid	Southern Europe, Northern Africa* [1]	Egypt[2]	Germany[3]	Denmark[4]	France[5]	Spain[6]
	29 cultivars	1 cult.	1 cultivar	4 cultivars	1 cult.	1 cult.
	%					
Lupanine	62.5–86.4	59.6	70	57.2–69.9	73.2	71.9
13-Hydroxylupanine	0–6.6	19.8	8	5.6–13	17.9	0.8
Angustifoline[7]		7.2	< 1	0.9–2.6	8.9	
Albine	5.2–25	<0.1	15**	7.4–10.1		6.5
Multiflorine	0–17.8	4.2	3	3.3–7.8		14.7
α-Isolupanine	0.4–12.5	<0.1	< 1	0.3–0.5		0.3
11,12-seco-12,13-Didehydromultiflorine		6.6	< 1	1.6–3.8		
13α-Tigloyloxylupanine		1.4	< 1	0.5–0.7		
13α-Angeloyloxylupanine		0.4	< 1	0.2–0.6		
Ammodendrine		0.8	< 1			
Sparteine		<0.1	< 1	0–0.7		
Tetrahydrorhombifoline[8]		<0.1	< 1			
Isoangustifoline		<0.1	< 1			

Alkaloid	Southern Europe, Northern Africa* [1]	Egypt[2]	Germany[3]	Denmark[4]	France[5]	Spain[6]
	29 culti-vars	1 cult.	1 cultivar	4 cultivars	1 cult.	1 cult.
			%			
5,6-Dehydrolupanine		<0.1	< 1			
13α-Hydroxymultiflorine		<0.1	< 1	1.0–1.5		
13α-Propyloxylupanine		<0.1	< 1			
13-Isobutyryloxylupanine			< 1			
13-Butyryloxylupanine			< 1			
13-Isovaleroyloxylupanine			< 1			
13-Angeloyloxymultiflorine			< 1			
13-Tigloyloxymultiflorine		<0.1	< 1			
Tetrahydrocytisine			< 1			
17-Oxolupanine[7,8]		<0.1	< 1	0.2–0.4		
Dihydroalbine		<0.1				
Dehydroangustifoline		<0.1				
Dihydromultiflorine		<0.1				
5,6-Dehydromultiflorine		<0.1				
11,12-Dehydrolupanine		<0.1				
3ß-Hydroxylupanine		<0.1				
N-Formylangustifoline		<0.1				
13α-Acetyloxylupanine		<0.1				
N-Formylalbine		<0.1				
15β-Hydroxy-17-oxolupanine[7]		<0.1				
13-(2-Methylbutanoyloxy)lupanine		<0.1				
Other alkaloids				1.8–2.5		
Total alkaloid content (mg/kg dry matter)	800–23,900	15,600	n.s.	248–470	123	30,700

*Turkey, Greece, Italy, Sudan, the Azores, Spain, Portugal, Morocco, Egypt, Ethiopia
[1] Múzquiz et al. (1994)
[2] El-Shazly et al. (2001)
[3] Wink et al. (1995). **Albine is not present in the commercial low-alkaloid cultivars of *L. albus* grown in Australia (Petterson, 1998)
[4] Als and Gade (1997)
[5] van Nevel et al. (2000) The seeds were imported from France. Only three alkaloids were identified.
[6] de la Cudra et al. (1994)
[7] (+)-Angustifoline is a minor alkaloid from the seed of white lupin. It was found in a concentration of 0.0096 % (Wysocka and Przybyt, 1993).
[8] Also isolated from the seeds by Mohamed et al. (1994)
Abbreviation: not specified: n.s.

Additionally, Wasilewko and Buraczewska (1999) studied the distribution of some alkaloids in seeds from white lupin grown in Poland. A similar pattern as shown in Table 2 with lupanine found in the highest proportion, followed by 13-hydroxylupanine, and with a lower amount of multiflorine was found in two cultivars. Lupanine was also found in the highest proportion in seeds from another cultivar analysed from two different harvest years. However, one year the alkaloids 13-hydroxylupanine and seco-12,13-didehydromultiflorine were found, the other hydroxymultifloine, hydroxylupanine and multiflorine.

No anagyrine was found in one bitter and one sweet strain of *L. albus* when measured by gas cromatography and mass spectrometry (Keeler and Gross, 1980). Nor was it found in seeds from a single strain of *L. albus* (Wink et al.,1995).

3.2.2 Lupinus angustifolius (narrow-leaved lupin)

A summary of the contents of total alkaloids in seed of high and low alkaloid containing varieties of narrow-leaved lupin grown in various countries is found in Table 3. Since there is not a general classification of whether the cultivars contain high or low levels of alkaloids or can be denoted as 'bitter' or 'sweet', the classification in bitter or sweet are based on the authors own description of the cultivars studied

Table 3. Total alkaloid content in the narrow-leaved lupin (*Lupinus angustifolius* L.) grown in various countries.

Cultivar	Grown in	No. of cultivars studied	Alkaloid content (mg/kg dry weight)	Analysis	Reference
'Bitter'	Poland, Portugal	3	14,400–19,600	GC-MS	Aniszewski et al. (2001)
'Bitter'	Denmark[1]	1	19,806–25,511	GC-MS	Christiansen et al. (1997)
n.s.	Poland	4	300–510	TLC	Wasilewko and Buraczewska (1999)
n.s.	Poland	3	740–6,670	Photometry	Niwinska (2001)
n.s.	Poland	8	250–1,000	Photometry	Sujak et al. (2006)
'Sweet'	Poland, Portugal	3	180–470	GC-MS	Aniszewski et al. (2001)
'Sweet'	Denmark[2]	2	700–1,918	GC-MS	Christiansen et al. (1997)
'Sweet'	Australia	n.s.	200[3]	n.s.	Petterson and Mackintosh (1994)
'Sweet'	Australia	n.s.[4]	30–470	n.s.	Petterson (1998)
'Sweet'	Australia	n.s.[5]	20–900	GC	Harris (1994)

[1] 'Bitter' variety originated from East Germany
[2] One 'sweet, and one intermediate 'sweet' variety from Poland
[3] Data from an Australian national grain legumes database, GRAILE
[4] Data on commercial crops from five zones in Western Australia from 1985–1992
[5] Data on commercial crops from eight receival points from Southern Western Australia from 1982–1991
GC: Gas chromatography
MS: Mass spectrometry
TLC: Thin layer chromatography
Abbreviation: not specified: n.s.

In a study of four cultivars of narrow-leaved lupin grown in Pol and the total alkaloid contents ranged from 300–510 mg/kg dry matter in the seeds when analysed with TLC. In all cultivars lupanine and 13-hydroxylupanine were found. In 2/4 the proportion of lupanine was higher than 13-hydroxylupanine. Additionally, seco-12,13-dide hydromultiflorine was found in one cultivar (Wasilewko and Buraczewska, 1999).

An overview of the alkaloid profiles of varieties of narrow-leaved lupin is shown in Table 4.

Table 4. Distribution of alkaloids (percentage) in seeds from narrow-leaved lupin (*Lupinus angustifolius*) grown in Australia, Denmark and Germany.

Alkaloid	Aust.[1] 1 cult. 'bitter'	Den.[2] 1 cult. 'bitter'	Den[3] 1 cult. 'bitter'	Aust.[4] 1 cult. 'sweet'	Aust.[1] 3 cult. 'sweet'	Den.[2] 2 cult. 'sweet'	Ger.[5] 1 cult. n.s.	Den.[3] 2 cult. 'sweet'
	%							
Lupanine	30–36	70–72	71	42–59	40–71	24–50	70	39–51
13-Hydroxylupanine	45–52	17–18	17	24–45	18–42	19–26	12	19–21
Angustifoline	8–16	5.5–6.4	6	7–15	9–16	1.9–5.6	10	3–4
Isolupanine		1–1.5	1	1–1.5		5.5–15	<1	6–14
Spartein		0.03–0.1				0.9–4.8	<1	3–5
Tetrahydrorhombifoline		2.5–2.9	3			0–1.6	<1	
Isoangustifoline		0.5–0.9				7.9–31	<1	8–11
17-Oxolupanine							<1	
Other alkaloids			2					4–10
Total alkaloid content (mg/kg dry matter)	15,000	19,800–25,500		n.s.	70–200	700–1,900	n.s.	n.s.

[1] Gremigni et al. (2001)
[2] Christiansen et al. (1997)
[3] Buskov (1995)
[4] Petterson et al. (1994)
[5] Wink et al. (1995)
Abbreviations: Australia: Aust., Denmark: Den., Germany: Ger., cultivar(s): cult, not specified: n.s

During the 1988/1989 season, analysis of total alkaloid content was made of samples from every truck that delivered lupin in one Australian receival point. The range of alkaloid levels was 10–520 mg/kg with a mean of 90 mg/kg. When studying the total alkaloid content of 46 individual trucks deliveries from one producer in Australia, the alkaloid level ranged from 10 to 380 mg/kg (Harris, 1994).

No anagyrine was present in seeds from two bitter and one sweet strain of narrow-leaved lupin (Keeler and Gross, 1980). No anagyrine was found either in seeds from a single strain of *L. angustifolius* (Wink et al. 1995).

3.2.3 Wild lupin species

As briefly mentioned, a number of wild lupin species have been reported to give rise to developmental/teratogenic effects in animals and possible humans. The alkaloids in these lupins are anagyrine, ammodendrine and N-methylammodendrine. Additionally, multiflorine has been suggested as a teratogen in *Lupinus consentinii* (Allen et al., 1983; Allen, 1998).

Table 5 summarizes available data on the alkaloid profile of seeds and leaves of these lupins.

Table 5. Alkaloid profiles of various lupin species suspected of giving rise to developmental effects in animals or man.

Alkaloids	L. caudatus 4 pop. Leaves	L. latifolius 1 pop. Leaves	*L. latifolius* 1 pop. Seeds	*L. formosus* 1 pop. Leaves	L. formosus 1 pop. Seeds	L. argentus 6 pop. Leaves	L. argentus 3 pop. Seeds	L. consentinii 1 pop. Seeds
	%							
Epilupinine								38
Gramine	0–14					0–2		
α-Isosparteine	0–6					0–54		
Sparteine	0–1				1	<1–19		
ß- Isosparteine						0–20	0–1	
Ammodendrine	<1–3					<1–2		
5,6-Dehydro-α-isolupanine	0–1					0–10	<1–8	
α-Isolupanine	<1–31			5		<1–67	<1–62	
Aphyllidine						0–23	0–1	
5,6-Dehydrolupanine	4–16					0–13	0–17	
Lupanine	7–35	10		15	17	<1–36	<1–37	
Aphylline	0–44							
Argyrolupanine						0–49	0–16	
11,12-Dehydrolupanine	0–2						0–1	
11,12-seco-12,13-didehydromultiflorine								3
N-acetylammodendrine				75				
3ß-Hydroxylupanine	<1–7	18			62	0–8	0–29	
Thermopsine						0–28	0–28	
Multiflorine					1			50
10,17-Dioxosparteine	0–2							
13α-epihydroxyisolupanine		29						
17-Oxolupanine	0–12	1			1		0–1	
Anagyrine	<1–64		1)			0–5	0–31	
Dihydroxyaphyllidin						0–6	0–12	
Dihydroxylupanine		3		5	5			
Dehydrolupanine	0–2	9			17		0–4	
7-Hydroxylupanine	0–20				3			
3,13-Dihydroxylu-		7						

Alkaloids	L. caudatus 4 pop. Leaves	L. latifolius 1 pop. Leaves	*L. latifolius* 1 pop. Seeds	*L. formo-sus* 1 pop. Leaves	L. formo-sus 1 pop. Seeds	L. argentus 6 pop. Leaves	L. argen-tus 3 pop. Seeds	L. consen-tinii 1 pop. Seeds
panine								
Other esters		20						
3-Angeloyloxylu-panine				5				
Hydroxyaphyl-lidine	0–3		90			0–9	0–27	

Alkaloid content <1 % is not shown unless there is a range in alkaloid content from low to a level higher than less than 1 %. Data are from Wink et al. (1995).
1) Gas chromatographic analysis of *L. latifolius* seeds "shown then to be very high in anagyrine" (Kilgore et al., 1981).
Abbreviation: number of populations studied: pop.

3.3 Other constituents in edible lupins

3.3.1 Macronutrients

Lupin seeds consist of an outer part, a seed coat (hull), and an inner part, cotyledon (splits, meats). The seed coats are thick in lupin seeds and comprise about 25 % of the seed weight in *L. angustifolius* and 15 % of the seed weight in *L. albus*.

The *protein* content in lupin seeds is 400 g/kg in the kernels of white and narrow-leaved lupin and 361 and 321 g/kg in the hulls from *L. albus* and *L. angustifolius*, respectively. The protein values from commercial dehulling of the seeds are 35 % from the cotyledon and 7–10 % for the hulls. The seeds are deficient in the *amino acids* lysine and methionine when compared to FAO standards.

The crude fat content is 91 g/kg and 58 g/kg in the hulls of *L. albus* and *L. angustifolius*, respectively. In the cotyledon the content is 114 g/kg in *L. albus* and 66 g/kg in *L. angustifolius*. The composition of the *oil* from *L.* albus is similar to edible oils e.g. from cruciferous plants e.g. rapeseed and mustard oils. This oil contains 1.5–2.7 % erucic acid (reviewed by Petterson,1998). The content of erucic acid in seeds is not regulated within EU but for edible fats and oils the maximum permitted erucic acid content is 5 % (Council Directive 76/621/EEC). The *crude fibre* content is high in the seed coat 149 g/kg in *L. angustifolius* and 103 g/kg in *L. albus*. The *carbohydrate* composition in the cotyledon differs from the hulls. The carbohydrates in the hulls are structural polysaccharides: cellulose, hemicelluloses and pectins whereas the main carbohydrates of the cotyledons are non-structural polysaccarides consisting of galactose, arabinose and uronic acid (Petterson, 1998).

3.3.2 Bioactive constituents with putative adverse effects

Bioactive compounds in plant based foods may be defined as *inherent non-nutrient constituents of food plants with anticipated health promoting/beneficial and or toxic effects when ingested* (Gry et al., 2007). Lupin seeds contain many other bioactive constituents than the *alkaloids,* which may be of health concern: *erucic acid, isoflavones, phytate, tannins, saponins, protease inhibitors, lectins* and *oligosaccarides* of the raffinose family. According to Petterson (1998) the amount of these constituents found in lupin seeds are low and not considered to have a negative influence on human nutrition. Especially, lupins have a low level of protease inhibitors and a negligible content of lectins compared to other legumes.

3.3.3 Mycotoxins

Outbreaks of a disease named lupinosis, a mycotoxicosis, have occurred primarily in sheep but also in cattle, goats, donkeys, horses and pigs that have eaten lupin seeds and other plant parts including lupin stubble infected with the fungus *Diaporthe toxica* Williamson, Highet, Grams & Sikisithamparam (previously known as *Phomopsis leptostromiformis* (Kühn) Bubak syn. *P. rossiana* (Sacc.) Sacc. & D. Sacc). The group of mycotoxins produced by this fungus is called *phomopsins*. Small quantities of phomopsins may be present as a latent infection on the lupin plants but toxins in quantities sufficient to cause disease is associated with the presence of visible lesions on the lupin stem, pods, or seeds. In seeds virtually all phomopsins produced are in discoloured seeds. The risk of using seeds infected with *Diaporthe toxica* can be largely avoided by visible inspection of the seeds and selection of samples of seeds with very small proportions of discoloured seeds, or by removing the discoloured seeds by commercial seed cleaning processes.

Experimentally, rats, mice, guinea pigs, rabbits, dogs, chickens and ducklings have been shown to be sensitive to these mycotoxins. Phomopsins exert their toxic effect by binding to microtubule proteins thereby inhibiting the formation of microtubules, that are essential for intracellular transport mechanisms and cell division. The main feature of lupinosis is severe liver damage which often results in death. Adrenal glands, pancreas, kidneys, rumen and muscles may also be affected. Lupinosis may cause abortion late in pregnancy of sheep and cattle and death of embryos in sheep early in pregnancy. Lupinosis has been reported in USA, Poland, Spain, New Zealand and South Africa but it is only in Australia it has been a disease of major importance because of the extensively use of lupin as fodder (reviewed by Allen, 1998).

In a worst case scenario, the daily intake of phomopsins was estimated to 8.5 ng/kg b.w./day. The estimation was based on the assumptions, that all white bread contained 50 % lupin flour, that humans eat 300 g white

bread daily equal to 200 g flour equivalent to 100 g lupin flour containing the maximum level permitted in Australia (5 µg phomopsins/kg). The daily intake is approximately 700 times lower than the NOAEL based on the effect on liver in sheep, the most sensitive animal species (Inger Thorup (2000), personal communication).

3.3.4 Allergens

There are limited reports (Hefle et al., 1994) that individuals known to be susceptible to one legume may also exhibit sensitivity to one or more other legumes, including peanuts. Additionally, in individuals sensitive to peanuts who react to other legumes the reactions to the other legumes are less severe than those seen to peanuts (ACNFP, 1996). The case described by Hefle et al. (1994) concerns a peanut sensitive child experiencing urticaria and angioedema after ingesting pasta containing sweet lupin seed flour from white lupin. Other cases of allergic reactions after ingestion of lupin seed are found in recent publications. A 17-year old woman with allergy to peanuts experienced acute asthma after oral challenge to lupin flour derived from white lupin (Kanny et al., 2000).

An adult man with no previous history of allery to legumes experienced angioedema, cough, dyspnoea and then anaphylactic shock after the ingestion of white lupin (Minore et al., 2001). Matheau et al. (1998) reported that an adult woman with known anaphylactic reactions to chickpea experienced a non-fatal case of anaphylaxis after intake of lupin as a snack for the first time. The species of lupin is not mentioned. Seven cases of serious anaphylaxis induced by lupin flour were reported by allergists in France in 2002. Four of the cases involved children and three adults. Overall 107 cases of food-induced anaphylaxis were registered in France in 2002 (Morisset et al., 2003). Lupin flour from white lupin was introduced in France in 1998. In Norway, a peanut-allergic patient reacted on several separate occacions with acute swelling of the lips, urticaria and rhinoconjunctivits against a certain brand of hot dog bread. Further study provided evidence that the patient serum reacted against lupin. The producer confirmed that the bread contained lupin that was not included in the ingredient list (Fæste et al., 2004). In UK a severe case of anaphylaxis in a woman was attributed to lupin flour used on onion rings in a restaurant meal. The patient had ten years before experienced a severe anaphylaxis after eating a peanut (Radcliffe, 2005). Three cases of allergy to lupin bran were recently reported in Australia. Two of the three patients had no previous history of food allergies (Smith et al., 2004). Smith et al. (2004) suggests that lupin should be added to the list of ingredients requiring mandatory allergy warning labelling in Australia. Lupin is not included in the list of potentially allergic ingredients that requires labelling according to a directive on food labelling that came into force in Europe in November 2004 (2003/89(EC) but its inclusion had

been recommended by the UK-based Institute of Food Science & Technology (Radcliffe 2005).

4. Exposure

4.1 Use of flour

As previously mentioned both cultivars with high and low alkaloid levels exist within the species *L. albus* and *L. angustifolius*. Seeds of bitter varieties of white lupin has been subject to a debittering process including cooking followed by soaking in water until bitterness has disappeared before they can be safely used as a food. The spiced, salted debittered seeds of *Lupinus albus* are used as a snack food in Southern Europe and also in Egypt (Marquez et al., 1991; Christiansen et al., 1999; Joray et al., 2007). Seeds from sweet lupin varieties can be eaten without prior processing. Lupin seeds derived from *L. angustifolius* are, however, more likely used as a food ingredients than to be consumed whole. Flour from the cotyledon can due to its high amount of protein (approx. 36–40 %) be used in protein enriched foods. Since the flour do not contain gluten it would be suitable for inclusion in foods for patients with coeliac disease (ACNFP, 1996). Low alkaloid containing *L. albus* seed flour and defatted flour has experimentally been used for replacing up to 15 % of wheat flour for bread making. Inclusion of lupine flour affected the volumes of the breads (Mubarak; 2001; Dervas et al. 1999). The defatted flour could substitute 10 % of wheat flour and produce an acceptable bread quality (Dervas et al., 1999). Substitution of wheat flour with up to 6 % of lupin flour had no detrimental effect on sensory properties of the bread (Mubarak, 2001). White lupin flour can substitute wheat flour at levels up to 5 % for baking white 'wheat' bread and has a greater potential for substitution of wheat flour than flour from narrow-leaved lupin. For substitution of up to 15 %, lupin flour is thought to be competitive with other grain legumes (Pollard et al., 2002). Full-fat lupin flour prepared from roasted, dehulled and milled lupin seeds (*L. albus* cv. Multolupa) could replace 12 % of the total fluor in two Chilean wheat bread types without affecting baking properties. By enriching bread with lupin, the content of available lysine increased 40 %, which is of nutritional importance since wheat protein is deficient in this amino acid (Ballester et al., 1988). Cookies containing up to 10 % of full-fat lupin fluor (*L. albus* cv. Multolupa) as replacement of wheat flour were evaluated as having a sensory quality similar to standard cookies. Inclusion of higher levels of lupin fluor (15, 20 and 25 %) affected the sensory quality and acceptability of the cookies (Wittig de Penna et al., 1987). Cookies prepared with up to 10 % flour from low-alkaloid seeds of *L. albus* had sensory quality and acceptability similar to or slightly better than cookies prepared without addition of lupin fluor. The sensory quality and acceptability of pancakes prepared

with up to 15 % of lupin flour from *L. albus* and dumplings filled with meat containing 5 % lupin flour were also good. Lupin hull from *L. albus* could be added to cookies in levels up to 10 %, in pancakes up to 5 % and in dumplings up to 5 % without much effect on sensory quality. However, addition of from 5–15 % of both lupin flour and lupin hull to minced meat had a dose-dependent negative influence on sensory quality. Minced meat containing 15 % lupin hull was rejected by the sensory panel (Górecka et al., 2000). Lupin flour can be incorporated at up to 50 % of the flour in biscuits (Kyle, 1994 cited in Dervas et al., 1999). Pasta including 15 % of *L. angustifolius* l flour is sold in Australia. In North America pasta supplemented with flour from *L. albus* has been marketed (Petterson, 1998).

Flour from hulls of *L. angustifolius* is rich in dietary fibre and could be used as an alternative to other sources of fibre e.g. high fibre bread or cereal bars (ACNFP, 1996). According to Petterson and Crosbie (1990) the hull is used as a fibre supplement in some breads in Australia.

4.2 Other uses

Lupin seeds could also be used to produce food ingredients such as protein isolates and lupin 'milk' (similar to soya 'milk') and could be used instead of soybeans in the production of a number of Asian fermented food like tempe and miso The traditional Indonesian food, tempe, is usually made by fermentation of soybeans. The content of alkaloids was reduced with 71 % in seed kernels from *L. angustifolius* (cv. Gungurru) soaked, boiled and fermented for the production of tempe compared to the content in the raw kernels (Fudiyansyah et al., 1995). Whole seeds could be used in soups and stew, sprouted for use in salads, or be used in stir fries or roasted as a snack food (ACNFP 1996, Petterson and Crosbie 1990. Experimentally, a protein isolate from *L. albus* has shown promising result as an alternative to egg white foams (Raymundo et al. 1998). Lupin flour from both *L. albus* and *L. angustifolius* showed emulsification properties. Overall *L. albus* showed better emulsifying properties than *L. angustifolius*, although *L. angustifolius* had better potential as a foaming agent (Pollard et al., 2002). Experimentally, lupin protein has been used for clarification of wine (Cattaneo et al., 2003).

4.3 Debittering of 'bitter' lupin seed

Only few data on the alkaloid content of bitter lupin species after debittering exist. The traditional debittering process includes cooking followed by soaking in water for days. Joray et al. (2007) describes a method for making savoury and sweet-coated snacks from 'bitter' *L. albus* following

the traditional Middle Eastern process by cooking at 80–90 °C for 30 min, soaking at salty water at 4 °C for 4 days with daily replacement of the water followed by dehulling of the seeds by hand the fourth day. The alkaloid content in such debittered seeds are reported to be approximately 500 mg/kg (R. Gross, Perth, 1984, personal communication, cited by Petterson, 1998). That the content of alkaloids can be experimentally reduced to levels lower than 500 mg was shown by Olver and Jonker (1997). Seeds of *L. angustifolius* with an alkaloid content of 900 mg/kg seeds were micronised (cooked by infra-red radiation at 80 °C for 40 seconds) without any effect on the alkaloid level. The alkaloid content was subsequently reduced to less than 200 mg/kg by soaking in water for 3 days. The water was removed and fresh water added twice daily. Mukisira et al. (1995) partly removed alkaloids from *L. albus* seeds by boiling in water for 1 h, steeping in running cold water for 12 h, and oven-drying at 65°C for 24 h. The average reduction in total alkaloid content (lupanine and 13-hydroxylupanine) after the above-mentioned process was about 40 % from a total mean alkaloid content in the seeds of 26 g/kg to 15.7 g/kg dry matter.

The alkaloid content in bitter *L. albus* seeds was studied at 0, 24, 48, 72 and 96 h under germination conditions with 8 h light and 16 h darkness per day. The study was terminated after 96 hours when the seeds developed leaves and photosyntetheic activity was apparent. The alkaloid content was 30.7 g/kg dry matter at 0 h, peaked after 48 h at 42.2 g/kg, and was reduced to 32.3 g/kg after 96 h. No new alkaloids were found during germination. Trial runs indicated that the germination was slower in total darkness (de la Cuadra et al., 1994). Sprouting of *L. angustifolius* (cv. Gungurru) seeds under natural lighting at 20–25 °C for 6 days reduced the total alkaloid content from 720 mg/kg to 160 mg/kg dry weight in the kernels, the de-hulled seeds (Dagnia et al., 1992).

4.4 Exposure estimation

No data are at present available from the Nordic countries on exposure to lupin seeds. In Danish retail shops and ethnic markets lupin seeds, snack foods made of lupin seeds, bread, waffles and frozen fast food (toast) with lupin flour have been observed. In Norway lupin flour from *L. albus* and *L. angustifolius* has been marketed and was detected in a special brand of hot dog bread, in other bakery products, pasta, vegetarian sausage and chocolate spread (Fæste et al., 2004; Løvik et al., 2004; Holden et al., 2005).

Estimates of daily intakes of alkaloids based on assumptions of lupin seed levels in various foods and maximum levels of lupin alkaloids of 200 or 500 mg/kg are shown in Table 6. The lupin alkaloid level of 200 mg/kg lupin seed flour, lupin kernel flour, lupin kernel meal and lupin

hulls is the maximum level permitted for all lupins (the genus *Lupinus)* in Australia (ANZFA, 2008).

A similar limit was recommended by the Advisory Committee on novel Foods and Processes in United Kingdom: maximum 200 mg lupin alkaloids in the seeds or derived lupin products from L. angustifolius (ACNFP, 1996). The alkaloid content of 500 mg/kg seed is considered to be representative for traditionally debittered high alkaloid containing seeds (R. Gross, Perth, 1984, personal communication, cited by Petterson, 1998).

Table 6. Estimated exposures to lupin alkaloids in the Nordic countries

	Alkaloid intake 1st Scenario	Alkaloid intake 2nd Scenario	Alkaloid intake 3rd Scenario	
		Adult (60 kg)		
Bread	6	15	-	mg/person
Pasta	3	7.5	-	mg/person
Snack	10	25	25	mg/person
Total daily intake	19	47.5	25	mg/person
Total daily intake	0.32	0.79	0.42	mg/kg bw
		Child (20 kg)		
Bread	3	7.5	-	mg/person
Pasta	2.3	5.7	-	mg/person
Snack	6	15	15	mg/person
Total daily intake	11.3	28.2	15	mg/person
Total daily intake	0.57	1.4	0.75	mg/kg bw

The first scenario (first column) is based on the assumption that an adult or young child ingest bread, pasta and snack containing lupin seed with the in UK recommended max. level of quinolizidine alkaloids (200 mg/kg).

In the second scenario again bread, pasta and snacks with lupin seed are ingested. However, the content of alkaloids is 500 mg/kg, a concentration that is not unlikely, if compared with the content in so-called 'sweet' lupin grown in Europe (Tables 1 and 3).

The third scenario is based on the assumption that an adult and a young child only eat snacks of traditionally debittered lupin seeds containing 500 mg alkaloid/kg lupin seed.

The estimation is based on the assumptions that an adult (60 kg) eats 300 g white bread consisting of 200 g flour containing 15 % lupin flour, 100 g pasta consisting of 15 % lupin flour, and 50 g lupin seeds as a snack. The assumed intake by a child (4–6 years, 20 kg) is 150 g white bread, 75 g pasta and 30 g lupin seed as a snack (Fagt, 2008. Personal communication).

5. Toxicity and feeding studies

5.1 Metabolism

5.1.1 Lupinus albus and Lupinus angustifolius

No animal data are available on absorption, distribution, metabolism and excretion of any of the quinolizidine alkaloids found in *L.albus* and *L. angustifolius*.

5.1.2 Other lupins

Craigmill et al. (1983) reported that significant quantities of anagyrine were found in milk samples taken from lactating goats fed daily doses of 227 to 340 g dried *L. latifolius* for 14 days. No quantitative data were reported.

Anagyrine was also detected in milk from goats for aging on *L. latifolius,* which contains high amounts of anagyrine. The goat gave birth to stillborn kids and/or kids with deformed legs. As also a dog fed the goats milk delivered deformed puppies, it is indicated that grazing on the anagyrine lupins gave raise significant amounts of anagyrine in the milk (Kilgore et al., 1981).

It is not known whether feeding of lactating farm animals with white or narrow-leaved lupins results in occurrence of lupin alkaloids or their metabolites in cow's, sheep's or goat's milk.

5.2 Acute toxicity studies

The acute oral LD_{50} of an alkaloid mixture from *L. angustifolius* (49 % lupanine, 39 % 13-hydroxylupanine, 10 % angustifoline, and 0.7 % α-isolupanine) was 2300 mg/kg b.w. in male Wistar rats fed prior to dosing and 2400 mg/kg b.w. in rats fasted for 16–18 hours before dosing. The acute oral LD_{50} for lupanine in fasted rats was 1700 mg/kg b.w. Intraperitoneal injection to fasted rats resulted in a LD_{50} of 180 mg/kg for lupanine and 200 mg/kg b.w. for 13-hydroxylupanine. Both oral and intraperitoneal administration of the alkaloids resulted in nervous signs with tremor and splaying and paddling of hind limbs within 1–16 minutes, followed by convulsions, cyanosis, collapse and death. Positive chronotropic effect was observed in some cases. Congestion of the liver and the lungs were

found in some rats post mortem. Rats that survived the treatment showed no further signs of clinical toxicity (Petterson et al., 1987).

Administration of extracts obtained from seeds of *L. angustifolius* and *L. albus* by gavage to AoBoy/Iiw mice of both sexes resulted in oral LD_{50} values higher than 4000 mg/kg b.w. Extracts from both lupin species contained approximately 10 % alkaloids. Fractionation of the extract from *L. angustifolius* with various organic solvents gave rise to fractions with LD_{50} values varying from 750–4000 mg/kg b.w. (Stobiecki et al., 1993).

Oral LD_{50} values for lupanine and sparteine in male EOPS Swiss mice were 410 and 220 mg/kg b.w., respectively. Intraperitoneal administration of lupanine and sparteine to the same mice strain led to lower LD_{50} values180 and 36 mg/kg b.w., respectively. All groups consisted of 10 animals. Identical symptoms were observed for the two alkaloids: trembling, tonic-clonic spasms followed by death from breathing arrest (Yovo et al., 1984).

5.3 Subacute/subchronic studies

Ballester et al. (1980) reported a toxicity study with rats,12 rats per group (sex and strain were not specified), fed a diet with 581 g seeds from *L. albus* per kg diet, or a control diet with casein for 112 days. The protein content was 20 %, and 0.3 % DL-methionine was added to the lupin diet. The total alkaloid content was 510 mg/kg. The weight gain of the rats fed the lupin diet was slightly lower than in the control group throughout the study but no statistically significant difference was found in body weight gain, feed intake and feed efficiencies measured during weeks 1–6. Data on these parameters from later in the experiment are not reported. No differences were seen in the relative organ weight of the liver, kidneys, heart, spleen and adrenals in the lupin fed group compared to the control group. No differences between the two groups were found after macroscopic or microscopic examination. However, only the histology of the liver, lungs and kidneys seems to have been studied.

Ballester et al. (1982) studied the toxic effects of a diet containing 51.8 g seed meal from *L. albus* (cultivar Multolopa) per 100 g diet (supplemented with 0.2 % DL-methionine), or a control diet fed to groups of 20 male and 20 female Wistar rats for up to 9 months. The lupanine content of the test diet was 0.025 % resulting in an estimated daily intake of lupanine of approximately 3.1 mg/rat. The content of other alkaloids seems not to have been studied. The protein content of both diets was 20 %. Half of the animals were killed after 24 weeks, the rest after 36 weeks. The feed intake was reduced in the lupin group at weeks 3, 4 and 5 for the male rats, and at weeks 3, 4, 6 and 7 for the female rats. At week 36 all blood samples from the lupin groups coagulated so haematology was not

performed. The lupin feed had no adverse effects on growth, mortality, haematology, or blood chemistry. Neither were the weight of the liver, kidneys, heart, spleen, brain and gonads affected. The histology of the liver, kidneys, brain, gonads and small intestine did not differ from the controls. However, the relative liver weight was statistically significantly reduced in the lupin group compared to the control group both after 24 and 36 weeks of dosing. The rats in this study were parents (F_0-generation) to the rats used for a multigeneration study (see section 6.6.)(Ballester et al., 1984).

Ballester et al. (1984) studied the effect of lupin feeding in a multigeneration study (F_1 and F_2) in Wistar rats. Twenty male and 20 female rats, were for 9 months fed a diet based on flour derived from seeds of *L. albus* containing 51.8 g seed meal from *L. albus* (cultivar Multolopa) per 100 g diet (supplemented with 0.2 % DL-methionine) and compared to control groups not receiving lupin. The lupanine content of the test diet was 0.025 %. The feed and lupanine content was the same as in the previously described study (Ballester et al.,1982). Five males and 10 females of the F_1 and F_2 generations were used for breeding by mating at the age of 12 weeks. Half of the animals were killed after 24 weeks, the rest after 36 weeks. Male rats fed the lupin diet had a statistically significantly higher body weight than control animals throughout the study. Such an effect was not observed in female rats. No effect of diet was seen on food consumption and food conversion efficiency. A general finding was that the relative liver weight was statistically significantly lower in the lupin-fed group than in control animals. Relative weights of the kidneys, heart, spleen, brain and gonads were unaffected by treatment. Neither there were seen treatment-related effects on haematological data: haemoglobin, haematocrit and total leucocytes or clinical chemistry: SGPT and SGOT. Gross examination at autopsy and microscopic examination of the liver, kidneys, brain, gonads and small intestine did not reveal any pathological changes that could be attributed to lupin feeding. It is possible to estimate the daily lupanine intake at week 6. The lupanine intakes during week 6 were for male rats estimated to 9.2 and 10.8 mg/kg b.w./day for the F_1 and F_2-generation, respectively. The corresponding lupanine intakes for females were 9.6 and 11.7 mg/kg b.w./day for the F_1 and F_2-generation, respectively.

Butler and co-workers (1996) conducted a 90-day feeding study in rats. Groups of 20 male and 20 female Sprague Dawley rats were fed a diet including 55.4 g lupin flour derived from *L. angustifolius* per 100 g diet. The control group received the lupin-containing feed and the treated groups the lupin-containing feed spiked with lupin alkaloids extracted from a bitter lupin cultivar (Fest). The profile of the major alkaloids in the extract was 44 % lupanine, 42 % 13-hydroxylupanine, 10–11 % angustifoline, and 1.0 % α-isolupanine. The control group received 50 mg lupin alkaloids/kg diet and the dosed groups 250, 1050 or 5050 mg/kg

diet. However, the alkaloids were not uniformly mixed within the diet as shown by a variation in the expected content from 60–116 % between samples of feed. The non-homogenity of the feed was especially problematic for the lowest dose. The stability of the alkaloids could not be accurately measured. Daily intakes were therefore calculated from the intended doses and not based on analytical data. The daily intakes for the first and the last days of the study (in brackets) are given The mean daily alkaloid intake for male rats were 6.6 (2.9) mg/kg b.w. for the control group, and 31.9 (14.4), 128.9 (59.9), and 597.1 (264.1) mg/kg b.w. for the dosed groups. The mean daily alkaloid intake for female rats were 6.5 (3.8), 33.4 (19.6), 135.3 (77.0), and 611.1 (364.0) mg/kg b.w., respectively. No death or behavioural changes occurred. Body weights and food intakes were not influenced by dosing. Water intake was increased in all treated female groups but was not statistically significantly different from the water intake in female controls. Significant increases in urine volume and decreases in the refractive indices were seen in all female groups from the 2 hour collection and in the 250 and 1050 mg groups from the 6 hour collection at 6 weeks and in the 2 and 6 hour collection in the lowest and medium dosed female groups at 13 weeks, whereas no changes occurred in the male groups. A dose-related decrease in red blood counts (RBC) were found in the male groups receiving 1050 and 5050 mg alkaloid/kg diet at days 42–49 but not day 90. Non-dose-related decreases in mean cell volume (MCV) were observed in all dosed male rats at days 42–49 and at day 90 whereas the MCV for female rats in the lowest and highest dose group were increased day 90. A dose-related decrease in haematocrit (HCT) values were observed in all dosed males at days 42–49 and 90, and in females days at 42–49. A decrease in haemoglobin concentration was found in males in the highest dose group at day 90. An increase in white blood cell count in female rats was observed day 90. Clinical chemistry measurement revealed no differences between dosed groups and control animals. Absolute brain, heart and liver weights were increased in females treated with 250 mg lupin alkaloid. Relative liver weights were statistically significantly increased in all dosed female groups but not showing a dose-response, and relative heart weights were increased in females in the low and medium dose groups. In the group receiving 5050 mg alkaloid/kg diet, 5/20 male rats had focal inflammatory lesions in the heart whereas none was seen in the control group. According to the authors such lesions are relatively common in the aged rats and are not considered to be of toxicological significance. In the highest dosed female group, 5/20 livers had small foci of altered hepatocytes either basophilic or larger pale-staining cells. Such foci were also seen in 1/20 females and 1/20 males in the 250 mg group and 1/20 males in the 1050 mg/kg group but none was observed in the control group. The authors state that foci of cellular alteration in the liver are increased by the administration of hepatocarcinogens in young rats. The increase of foci in

young rats suggests a compound-related effect and that longer exposure to the alkaloid would elucidate the nature of this effect.

Robbins and co-workers (1996) studied groups of 20 male and 20 female Sprague Dawley rats fed diets containing lupin alkaloids at dose levels of 0, 100, 330, 1000 and 5000 ppm for 90 days. The equivalent calculated average daily intakes of lupin alkaloids were 0, 8.5, 28.1, 85.3 and 410 mg/kg b.w. for males and 0, 10.2, 34.0, 99.4 and 486 mg/kg b.w. for females The lupin alkaloids were extracted from Fest, a bitter variety of *L. angustifolius*. However, no information on the exact alkaloid profile is given. The following parameters were examined: body weights, food intakes, haematology, serum chemistry, organ weights of liver, kidneys, heart, adrenals and brain, and histopathology of liver, heart and bone marrow from all animals together with any tissue that appeared abnormal at autopsy. No deaths or changes in behaviour attributable to alkaloid ingestion were observed. A statistically significant lower ($P < 0.05$) mean body weight was observed in the 5000 ppm group compared to control animals both for male and female rats. Consistently lower mean body weights were found in the 1000 ppm male and female groups but not reaching statistical significance. At the end of the experiment, body weights were significantly reduced in males ($P \leq 0.001$) and females ($P \leq 0.01$) given the 5000 ppm dose level, and also in females ($P \leq 0.05$) given 1000 ppm lupin alkaloids. Food intakes of both sexes were significantly affected in the two highest dose groups where an initial drop in food intakes was followed by reduced intakes (90–95 % of control levels). Some changes in haematological parameters were seen but no dose-response relationship was observed. In female rats of the 5000 ppm group red blood cell count (RCB), haemoglobin (Hb) and haematocrit (Hct) were reduced after 12 weeks of dosing but not after six weeks. A reduced RBC and Hct were also seen in the 100 ppm group but not in the intervening groups. Minor changes in serum chemistry were found. Thus, in females receiving the highest dose protein was elevated both after 6 and 12 weeks. Relative liver, kidney and brain weights were increased and absolute heart weight was reduced in the male rats at the highest dose level. Relative liver, heart and brain weights were increased and absolute kidney weight reduced in the female rats at the highest dose level. At the 1000 ppm dose level absolute liver weight was reduced, and relative kidney and brain weights were increased in the females. No treatment related effects were observed at the histopathological examination. The authors suggest a NOAEL of 330 ppm for the lupin alkaloids based on data of the reduced body weights and food intakes. However, they speculate that the changes may be caused by the antipalatability of the higher doses of lupin alkaloids and consider, therefore, a NOAEL of 1000 ppm as more appropriate.

5.4 Chronic/carcinogenicity studies

No chronic studies with the two lupin species or their alkaloids were available.

5.5 Genotoxicity

No data on genotoxicity of the alkaloids or extracts of white or narrow-leaved lupin are published.

Unpublished data on *in vitro* studies on isolated alkaloid preparation from *L. angustifolius* showed negative mutagenic effects in normal and stimulated cultures of *Salmonella typhimurium* (Petterson (1998) refers to a report from BIBRA 1986).

5.6 Reproduction/developmental toxicity

Four groups of 4-week-old male Sprague-Dawley rats, 20 rats per group, were for nine weeks fed control diets containing 3 or 10 % casein, or diets with 27 % untreated or 27 % debittered lupin seeds (*L. albus)*. The lupin seed diets contained 10 % protein supplied from the lupin seeds. The 3 % casein diet was chosen to serve as a control with regard to body weights for the two groups fed lupin seeds. Lupin seeds were debittered by boiling for 30 min followed by soaking with frequent changing of the water until the bitter taste was virtually removed. The alkaloid content was not reported. The data seem not to have been analysed statistically. The final average body weights were 98 g and 223 g for the 3 % and 10 % casein groups, respectively, and 85 g and 124 g for the groups fed untreated or debittered lupin seeds, respectively. The weekly food intake was 38 g for the 3 % casein group, 82 g for the 10 % casein group, 31 g for the untreated lupin group, and 37 g for the debittered lupin group. The poor growth of the rats fed the lupin diets were concomitant with the reduced feed intake. No deaths were reported. The group of rats receiving untreated lupin diet had smaller seminal vesicles than the other groups. Microscopic examination of the seminal fluid and smears of testicular tissue of rats fed the untreated lupin diet showed only few spermatozoa of which some were abnormal, inactive or dead. Testes of rats fed the debittered lupin diet also contained less spermatozoa than the group of rats fed 10 % casein but the spermatozoa appeared normal in shape and activity. The overall appearance of the testes of rats fed the 3 % casein diet was normal but some spermatozoa were deformed or immobile. Histological examination of the testes of rats fed untreated lupin seeds showed atrophy of the seminiferous tubules and they contained primary spermatocytes, Sertoli cells and many degenerate or multinucleated coalescing sperm at-

ids. The appearance of th e testes of the other three groups was normal and in com parable stage s of sper matogenetic develop m ent and all had mature and norm al sper matozoa in their epididymides. Testic ular homogenates were incubated with [4-^{14C}]progesterone for 3 h at 37 ^{o}C and the recoveries of radioactivit y in four i solated fractions: polar fraction; testosterone, 17-h ydroxyprogestorone and related com pounds; androstenedione an d related com pounds, a nd pro gesterone were studied. The authors pay special att ention to the fact , that rats fed the untreated lupine accumulated more radioactivity (13.8 %) in the androstenedione area than all the other groups. However, the group receiving 3 % casein accu mulated 10.0 %, co mpared to 3.2 % and 3.8 % in the groups fed debittered lupin, and 10 % casein, respectively (Tannous and Nayfeh, 1969).

In a study by Ballester et al. (1984) (described in section 6.3), sev eral generations o f Wi star rats (F_1 and F_2), 20 male and 20 female rats p er group, were fed a diet based on flour d erived from *L. albus* seeds (51.8 g per 100 g diet) supplemented with 0.2 % DL-methionine for 9 months. The lupanine content of the diet was 0.025 % . The lup anine intak es during week 6 w ere estimated to be b etween 9 and 12 mg/kg bw/d ay for the F_1 and F_2 generations.The protein contents in the lup in and control diets were 20 %. The r esults from th e F_0-generation were reported by Ballester et al. (1982). The F_2- and F_3-generations were obtained by mating five males and ten females of the F_1 and F2 generation at 12 week s of age. Treatment continued during the mating p eriod of 3 we eks, pregn ancy and lactatio n, and dams con tinued on th eir d iet for up to 9 month. Litters were ran domly culled to eight on day 5. Parameters studied were the percentage of females with litters, n umber o f pups in each l itter and th e tot al weight of th e litter days 1, 6, 16 and 21. F ertility was similar in both generations and th e growth of the pups fed lupin was similar to control animals.

5.7 Feeding studies

Sparteine and lupanine have an affin ity to cholinergic receptors of nicotinic type shown both *in vivo* and *in vitro*. At doses near the lethal doses the effects of the two alkal oids are ni cotinic-like. At lower doses a slight sedative action o n the C NS are found in behavioral tests for explorin g behaviour and locomotor activity in mice (Yovo et al., 1984).

5.7.1 Feeding studies in rats

Ballester et al (198 0) fo und a ver y l ow weight gain in rats (21– 23 da ys old) fed a diet with lupin seed from *L. albus* (290 g/kg diet) containing 10 % protein for 28 da ys compared to a control gro up fed a casein diet. The total alkaloid content of t he seeds was 510 m g/kg. Addition of 0.1 DL-methionine to the lupi n diet im proved the weight gain to a level com pa-

rable to the casein diet. Addition of D L-methionine levels to 0.2–0.3 % did not further improve weight gain compared to the 0.1 % level.

Groups of 8 male Hooded Lister rats were for 10 days fed *ad libitum*: a non-protein control diet, a control diet with lactalbumin, a diet with lupin seed meal (360 g/kg diet) from a 'sweet' variety of *L. angustifolius* obtained from the Grain Pool of Western Australia, and a diet with lupin seed meal supplemented with essential amino acids upto target requirements for rats. All diets containing protein had a 10 % protein content. The alkaloid content of the lupin diets was not given. The unsupplemented lupin group showed a reduced weight gain, a reduced gain/feed ratio, and a reduced relative lipid content (total lipid content per 100 g dry b.w.) compared to the control group. Animals in both lupin groups had lower dry body weights and dry body nitrogen than control animals whereas the net protein utilisation was 47 % and 65 % for the unsupplemented and the supplemented lupin diets, respectively, and 97 % for the control group (Rahmann et al., 2000).

A parallel experiment with restricted diet (7g diet/day/rat) for 10 days was reported in Rahmann et al. (1996 and 2000). The study was made up of a non-protein-group of 8 rats, a control group of 20 rats, a unsupplemented lupin group of 16 rats, a supplemented group of 20 rats, and a group of 4 rats receiving a lupin seed protein fraction (seed protein extracted at pH 7.0 with water and insoluble after dialysis at pH 7.0). For both lupin groups, the weight gain was reduced, the urine content of nitrogen and urea were increased, and wet and dry carcass weight were reduced. Data from the supplemented lupin group were closer to control values than data from the group not being supplemented. The final median body weight was statistically significantly reduced in the group fed unsupplemented lupin compared to the control group. The relative dry organ weight (dry organ weight/100 g dry b.w.) of kidneys was increased in the unsupplemented lupin group and the relative intestine weight was increased for the supplemented and unsupplemented groups. The serum urea was increased and albumin level was reduced in the supplemented lupin group. These parameters were not tested for the unsupplemented group. Rats receiving the lupin protein fraction had the most severe changes compared to controls. These changes included in addition to the differences described for the lupin diets, also reduced water intake, increased urine output, increased water and protein content, reduced relative lipid content, and reduced spleen weight. Microscopic changes of the liver including hydropic degeneration, fatty changes and focal accumulation of polymorphonuclear cells, pycnotic hepatocytes in affected lobules and fibrosis confined to the peripportal areas, and bile ductular proliferation were found in groups fed supplemented and unsupplemented lupin diets, and the lupin seed protein fraction. Severe fibrous changes and bile ductular proliferation was found in the latter group. No microscopic changes were seen in the kidney and small intestine of the lupine groups

compared to control animals. Rah mann et al. (1996) suggest a link between fibrosi s in the live r and protein deficiency but also sp eculate whether i.e. Kuppfer cell proliferation may be caused by alkaloi ds. The authors mention that the n ature of the proteins in the protein fraction remains obscure but suggest based om the SDS-PAGE pattern that they are lectins (Rahmann et al. 2000).

In another study , using ra w lupin seed (*L. an gustifolius)* as the onl y source of protein in a diet with 10 % protein resulted in a great reduction in food intake, weight gain and net protein utilisation (30–35 %) in eight rats fed the d iet for 10 day s com pared to four contr ol rats fed a control diet with egg albumin. The alkaloid content in the lupin seed was low but the concentration was not given . Supplementation with am ino acids to target requirements for rats increased the food intake to levels higher than the control diet and increased the net protein utilisation for the lupin diet to 53 %. W hen four rats were pa ir-fed with suppl emented lupin diet, weight gain was lower in the group fed supplem ented lupin seed compared to four control rats (Yen et al., 1990).

Jéscai et al. (1986) studied the eff ect of inclusion of 26.2 % of bitter white lupin seeds in the di et as the sole protein sour ce to two groups of growing male Wistar rats, 10 animal per group, for 21 days. In one group the lupin diet was supplemented with 0.3 % DL-methionine. A control group received extracted soy bean instead of lupin. T he content of crude protein in the diets was for all groups 9 .5 % The total alkaloid cont ent in the lupin die ts wa s 2 g/kg diet. In another experiment by Jésc ai et al. (1986), five groups of male Wistar rats, 10 animals per group, were fed a control diet consisting of extracted soybean, or diets containg mixtures of soybean or bitter or sweet white lupin seeds resulting in a total alkaloid content of 70 0, 500, 80 or 50 mg/kg diet for 60 days. The crude protein content of the diets was about 15 %. The feed was su pplemented with L-lysine and DL-m ethionine. The data seem not to ha ve been stati stically analysed. Feeding of rats with diets w ith a content of 2 g alkaloid/kg diet, resulted in poor protein utilization, ina ppetence, loss of body mass and occasionally deaths occurred. The bo dy weight gain was on average 1 g/rat/day in the group fed lupin seeds supplem ented with DL-methionine whereas the body weights decreas ed on average 0.5 m g/rat/day in the unsupplemented group. Histopathological examination showed lipid granules in the livers of the groups given lupin. The bl ood urea increased in the lupin groups and t he total am ino-acid N in blood decreased. The amino acid content of the liver protein s, especially the sulphur- bearing amino acids, was lower in the groups fed the lupin diet co mpared to the control group.

No deaths, clinical findin gs, or hist opathological changes in the livers were reporte d in the expe riment where rats were dosed with lupin seeds for 60 days. Blood urea concentration was lowest in the control group and higest in the groups receiving 700 and 500 m g alkaloid/kg diet. No sig-

nificant differences were found between groups concerning total amino acid-N concentrations in blood. The liver proteins of the rats fed lupin seeds were poorer in sulphur-bearing aminoacids.

In a feeding study, 27 male Hooded Lister rats were fed a diet containing 320 g lupin seed from *L. angustifolius* per kg diet for up to 700 days. The alkaloid content in the seeds was not analysed. Thirty-nine rats were fed a control diet containing lactalbumin, maize starch, potato starch, glucose and maize oil. Twelve lupin-dosed rats were killed after 250, and 15 after 700 days of dosing. No deaths occurred in the rats that received lupin seeds and one control rat died between day 500 and 700. The body weights were similar for dosed and control rats day 30 but statistically significantly ($P \leq 0.01$) reduced for the lupin fed rats after 250 and 750 days. Also the dry body weight was reduced after 250 and 750 days. No changes were seen in the carcass contents of water, lipid and protein. Apart from higher relative dry weights (dry weight of organ divided by dry b.w.) of caecum and colon, relative weights of the stomach, small intestine, pancreas and gastrocnemius muscles were comparable between lupin-dosed and control rats. No data on other organ weights or toxicological parameters were recorded (Grant et al., 1995).

In another feeding study by Grant et al. (1993), 48 male Hooded Lister rats were fed a diet with 320 g *L. angustifolius* seed per kg diet for up to 800 days. The alkaloid content was not measured. Eight rats were sacrificed day 30, 4 rats day 100, 8 rats day 150, 8 rats day 350, 5 rats day 600, 10 rats day 700, and 5 rats day 800. The feeding was restricted to what in a previous study had been eaten of a soy bean diet at the same age. The control group (120 rats) had free access to a diet with lactalbumin, maize starch, potato starch, glucose and maize oil. At day 200 the rats weighed on average 50–70 g less than controls. The weight gain from 200–800 days was very similar between groups. No deaths occurred in the group fed lupin seeds. The focus of the study was on pancreatic enlargement, and lupin seeds had no effect on pancreas. No data on other organ weights or toxicological data are presented.

5.7.2 Feeding studies in chickens

Olkowski et al. (2001) studied the effect of feeding groups of male broiler chickens, 16 animals per group, a control diet with 35 % soybean meal, or test diets with 40 % raw, 40 % autoclaved or 35 % dehulled lupin seed meal derived from *L. angustifolius* cultivar Troll for 21 days. Chemical analysis of the lupin seeds showed that the total alkaloid contents were below 0.01 % when studied by the method of Ruiz (1977). No detectable levels of the mycotoxins, ochratoxin A, nivalenol and deoxynivalenol, T2 and diacetoxyscirpenol, were found when analysed by thin layer chromatography. Decreased food intake and growth rate were observed in all chickens fed the lupin-based diet. During the first week of exposure, four

birds fed raw lupin seeds showed clinical signs of acute toxicity including leg weakness, lack of coordination and torticollis. Two birds in this group died and two were euthanized when found in moribund condition. During the second week of exposure three chickens in the group fed raw lupin seeds started to show mild signs of wing paralysis and at the end of the study two of the three affected birds were unable to hold their wing in the normal upright position for more than a few seconds. Birds showing signs of acute toxicity were subjected to electrocardiographic examination and compared to four normal birds from each group and remaining birds were examined day 21. Lower heart rate, bradycardia, was observed in the intoxicated chicken compared to control animals. No other forms of cardiac arrytmias were observed. No difference in heart rate was observed at the end of the study. In the groups fed lupin, skeletal deformities were shown in 3/16 birds fed raw lupin, 3/16 dehulled lupin and in 1/16 fed autoclaved lupin. Affected chickens showed limb deformities, crooked sternum and mild scoliosis in the thoracic vertebrae region at necroscopy. Statistically significantly higher liver cytochrome P-450 level were found in chicken fed lupin diets compared to controls. Apart from the skeletal deformities, post mortem examination did not show other treatment-related gross pathological changes.

5.7.3 Feeding studies in sheep and lambs

No developmental effects were described in lambs when the ewes had been fed on a diet of oat stubble supplemented with different quantities of sweet lupin seeds (*L. albus*) during the last 6 weeks of pregnancy and the first 6 weeks of lactation. The doses of lupin seeds were 0, 200, 400 or 600 g/animal/day. The alkaloid content in the seeds is not determined. Supplementation with lupin reduced the body weight loss of ewes during lactation. No significant differences between supplementary groups were observed in terms of lamb birth weight or the lamb growth rate up to 6 weeks of age (Brand et al., 1997).

5.7.4 Observations in pigs

The production records from a research station studying the performance of pigs fed lupin seeds were reviewed. Eight generations of pigs and a total of more than 1100 litters had been fed grower or finisher diets containing from 10–40 % of *L. angustifolius* seeds, and sow diets containing 10–30 %. Alkaloid levels in the diets were estimated to range from 10–80 mg per kg. No indications of teratological effects or significant lesions in slaughtered sows were found. The production data were comparable to production data from pig industry that did not use lupin diets (Petterson, 1998).

5.8 Summary of toxicity and feeding studies

Acute effects with symptoms from the nervous system after oral intake of lupin alkaloids have been descibed in laboratory animals like rats and mice. The acute oral LD_{50} for lupanine in fasted male Wistar rats was 1700 mg/kg b.w. No data on the acute oral LD_{50} level of mixtures of alkaloids from *L. albus* are available but since lupanine constitutes 60–86 % of the total alkaloid content data on this substance give some indication of the effects of *L. albus*. Dosing male rats with mixtures of alkaloids from *L. angustifolius* (49 % lupanine, 39 % 13-hydroxylupanine, 10 % angustifoline, and 0.7 % α-isolupanine) gave rise to an acute oral LD_{50} of 2300 mg/kg b.w. or 2400 mg/kg b.w. in fasted male rats and in rats fed prior to dosing, respectively.

Five *subacute/subchronic studies* – all in rats – have been performed: three with seeds from *L. albus* and two with seeds from *L. angustifolius*. In two of the studies with *L. albus* seeds diets with 58 % (0.05 % alkaloids in the diet) or 52 % (0.025 % lupanine in the diet) lupin seeds were given to rats for 112 days or nine months, respectively. The only compound related effects were seen in the latter study where reduced relative liver weights were shown. In the third study with *L. albus*, a multigeneration study in rats, the animals were given either 52 % (0.025 % lupanine in the diet) lupin seeds or casein in the diet for 36 weeks. The only lupin diet related effects seen were higher body weights in male rats and lower relative liver weights than in controls. In the first of the two studies with *L. angustifolius* all four groups of rats were fed 55 % lupin seeds in diet spiked with 50, 250, 1050 and 5050 mg/kg diet of total lupin alkaloids for 90 days. Most remarkably, an increase in small foci of altered hepatocytes was seen in the three highest dosed groups, and therefore the authors suggest a compound-related effect, but as the alkaloids were not uniformly mixed in the diet, the study is rather inconclusive. In the other study with *L. angustifolius* rats were fed 0, 100, 330, 1000 and 5000 mg lupin alkaloids (extracted from a bitter variety of *L. angustifolius*) per kg diet for 90 days. The authors suggested a NOAEL of 330 mg/kg food based on reduced body weight and food intake data (but also higher relative and absolute organ weights were seen at the two highest doses).

Eight *feeding studies* in rats were considered, three with *L. albus* and five with *L. angustifolius* seeds. In the three studies in rats with *L. albus* seeds for 21, 28 or 60 days with 26 % (0.2 % total alkaloids in diet), 29 % (0.015 % in diet) or mixtures of lupin seeds corresponding to up to 0.07 % total alkaloids in diet, respectively. The major findings were reduced weights in treated animals compared to controls. In the study with lupin seeds diet containing 0.2 % totel alkaloids (with lupin seeds as the only protein source and without DL-methionine), also lipid granules in the liver and occasionally death occurred. In the three of the five rat studies with *L. angustifolius* seeds rats were given up to 36 % lupin seeds (alka-

loid content unknown) for ten days, which resulted in reduced weight, generally with concomitant reduced food intake. In the two last studies rats were fed up to 700 or 800 days with 32 % lupin seeds (alkaloid content unknown) in the diet. Beyond reduced weights high relative weights of caecum and colon were seen in the treated animals compared to the controls.

There are also feeding studies available in *chickens* and *sheep*. In a 21 day feeding study four groups of broiler chicken were given diets containing 40 % raw, 40 % autoclaved or 35 % dehulled *L. angustifolius* seeds (less than 0.01 % alkaloids in the diets) or 35 % soybean as control. All the treated groups had reduced body weight and food intake compared to controls and 3/16 rats fed raw, 3/16 rats fed dehulled lupin seeds and 1/16 rats fed autoclaved lupin seeds had skeletal deformities. *Ewes* were fed 0, 200, 400, or 600 gram *L. albus* seeds (content of alkaloids unknown) per animal six last weeks of pregnancy and six first weeks of lactation. No developmental effects in terms of birth weights or growth rates were observed in the *lambs* of up to six weeks of age.

Large scale comparison of production data from a research station feeding *pigs* with up to 40 % *L. angustifolius* seeds with up to estimated 80 mg alkaloids per kg diet with production data from pig industry not using lupin seeds in the diet, did not indicate teratogenic effects or significant lesions in the lupin seed fed pigs.

Two *reproductive/developmental toxicity* studies have been performed – both in rats.

In the first study rats were fed 3 or 10 % casein, 27 % untreated ("higher lupin alkaloid content") or 27 % debittered ("lower lupin alkaloid content") *L. albus* seeds in the diets. The two lupin diets contained 10 % protein corresponding to the 27 % lupin seeds

Poor growth concomitant to reduced feed intake were seen for the two lupin fed groups. The untreated lupin seed had a detrimental effect on rat testes showing atrophy of seminiferous tubules and some abnormal spermatozoa of which several were abnormal or death.

In the other rat study several generations of rats were fed diets containing 52 % *L. albus* seeds (0.025 % lupanine in the diet) or casein as control. Fertility was similar in both generations and the growth of the pubs fed lupin seeds was similar to the controls.

6. Human data

6.1 Metabolism

The distribution of lupanine and 13-hydroxylupanine was studied in 11 subjects, 4 'poor' metabolizers and 7 'extensive' metabolizers to observe whether the molecules undergo polymorphic metabolism by N-oxidation at the 1 position of the molecule as known for another quinolizidine alkaloid, sparteine. The two alkaloids were studied separately by oral administration of 10 mg of each. For both alkaloids the half-lives were 6-7 h with 90-100 % being recovered as unchanged alkaloid in the urine. In one 'poor' and one 'extensive' metabolizer 34 and 14 % of 13-hydroxylupanine, respectively, was recovered as lupanine. No effect on heart rate or blood pressure was observed. The data indicate that no metabolism by N-oxidation takes place and that the administered doses are too low to cause any acute toxicological effect (Petterson et al., 1994).

6.2 Acute toxicity

Some human cases of acute intoxication with high alkaloid containing lupin seeds have been described in the literature. Two adult women in Australia and Canada were intoxicated after ingestion of raw or inadequately prepared high alkaloid containing lupin seeds. In the Australian case the seeds derived from *L. albus* and in the second case the lupin species was not identified (Lowen et al., 1995; Smith, 1987). Neither was the lupin species identified in a case where a young Spanish man was intoxicated after drinking half a liter of water used for debittering of lupin seeds (Marquez et al., 1991). Quantitative data are reported describing an intoxication in an adult man after ingestion of raw white lupin seeds containing about 2 % alkaloids. The estimated intake was approximately 23–24 mg lupanine/kg b.w. corresponding to an intake of total alkaloids of approximately 31–46 mg/kg b.w. Additionally she reviewed a case where a female (55 kg) ate 70–80 g of raw dry seeds of white lupin giving rise to an assumed intake of total alkaloids of 25–29 mg/kg b.w.. In adults symptoms arise 1–14 hours after ingestion and include dry mouth, muscular weakness, disturbed balance, sweating, palpitation, blurred vision, mydriasis (e.g. dilated pupils), urine retention, gastric and intestinal trou-

bles and abundant ventricular extrasystoles (Schmidlin-Meszaros, 1973; Lowen et al., 1995; Marquez et al., 1991).

Three lethal cases in children have been reviewed. The deaths of three children were reported after ingestion of 25–30 g lupin seeds in a 10-year-old boy of 30 kg, a 1½ years old child (9 kg) died after eating 5–10 g seeds, and a 17-month-old child (8 kg) died after eating 5–10 g seeds. The assumed deadly intake of total alkaloids varied from (1) 11–25 mg/kg b.w. in these children. In these three cases in children symptoms were mydriasis, seizures, cyanosis and respiratory arrest followed by death within three hours (Schmidlin-Meszaros, 1973).

A monthly intake of up to 3 g lupin seeds for eight years was suggested as the possible cause of a rapidly progressive motoneuron disease in a woman. An incomplete recovery was seen within two months after stopping the intake of the seeds. The authors suggest that the seeds were from *L. albus*. The content of alkaloids was not mentioned (Agid et al., 1988).

6.3 Developmental toxicity

Kilgore et al. (1981) published a case suggesting a link between severe bone deformities in the arm and hand of a baby boy whose mother had drunken goat's milk regularly throughout her pregnancy. *L. latifolius* foliage and seeds, which are high in anagyrine, formed the principal forage for goats in the area. Additionally, the goats had given birth to stillborn kids or kids with deformed legs. Furthermore, a dog that had been fed goat's milk during pregnancy had likewise deformed puppies. None of these deformed animals had been avaliable for study. The same boy's condition at birth and at the age of 5 years is reported by Ortega and Lazerson (1987). Other develomental defects seen in this child were a persistent azygous vein continuation of the inferior vena cava, and severe anemia. Further studies revealed the lack of erythrocyte precursors series in the bone marrow. Both the boy's parents, his siblings, and other family members had normal complete blood counts and none had congenital anomalies. The timing of the toxic insult is pinpointed to between the fifth and sixth weeks of embryonic development.

7. Discussion and conclusions

It seems that humans, especially children, are much more sensitive to acute toxic effects from lupin alkaloids than experimental animals (mainly rats studied). The acute oral LD_{50} -values for lupin alkaloids in rats are found in the range of 1700–2300 mg/kg b.w. In comparison, an adult man became severely intoxicated after ingestion of lupin seeds with approximately 2 % total alkaloid from *L. albus* estimated to an intake of approximately 31–46 mg/kg b.w. of total alkaloids and a woman became severely intoxicated after the intake of 25–29 mg of total alkaloids/kg b.w. In small children the deadly dose of alkaloids from bitter lupin seeds was estimated to 11–25 mg kg/b.w. in small children equivalent to doses as low as 5–10 g seeds. In the cases with small children the actual lupin species responsible for the deadly intoxications was not mentioned but it is most likely that it was bitter seeds from *L. albus* that were involved. This indicates that the rat might not be a good animal model for studying possible toxic effects in humans from foods containing lupin seeds.

Five subacute/subchronic (including two 90 day studies) and eight feeding studies available, all in rats, are reviewed. In most cases, rats fed lupin seed diets (*L. albus* or *L. angustifolius*), possibly spiked with lupin alkaloid extracts, did show reduced body weight, often with concomitant reduced food intake, compared to the controls. In one of the two 90 days studies an increase in small *foci* of altered hepatocytes was seen in the three highest dose groups of rats fed up to 5000 mg lupin alkaloids per kg diet and the authors suggested a compound related effect. But as the alkaloids were not uniformly mixed in the feed, the study is rather inconclusive. In five other studies the alkaloid contents in the diets are not given and only in one study the alkaloid profile is given.

All together, these rat studies are only of limited value for the prediction of possible toxicity in humans caused by exposure to lupin alkaloids, also taking into consideration the apparent very big difference in sensitivities between humans and rats when comparing acute toxicity.

With respect to reproductive and developmental toxicity there are only few studies available (see Sections 6.6 and 6.7). In a nine week rat study with 27 % *L. albus* seeds, untreated or debittered (alkaloid content not given), histological examination of the testes of the rats fed untreated lupin seeds showed atrophy of the seminiferous tubules, content of primary spermatocytes, Sertoli cells, and many degenerated or multinucleated coalescing spermatids. The appearance of the testes and the spermatozoa and the spermatogenetic development of the controls and the group fed debittered lupin seeds were normal. As the diets were very unbalanced, it is questionable whether the results are related to the lupin

alkaloids. In a multigeneration study, several generations of rats were fed 52 % *L. albus* seeds, corresponding to 0.025 % lupanine in the diet. During week six, the estimated intakes of lupanine were between 9 and 12 mg/kg b.w./day in the F_1 and the F_2 generation. Fertility in both generations and growth of the pups were similar to the controls. A feeding study with broiler chickens fed *L. angustifolius* seeds for 21 days resulted in skeletal deformation in 3/16, 3/16 and 1/16 chickens fed raw, dehulled and autoclaved lupin seeds, respectively.

Several non-edible lupin species have been connected with developmental effects in domestic animals. When *L. laxiflorus, L. caudatus* and *L. nootkatensis* were eaten by pregnants cows, these lupins have caused the congenistal effect "crooked calf disease". The quinolizidin alkaloid anagyrin is belived to be the teratogenic constituent in these lupins. A case story indicates that anagyrine also can be suspected to be a human teratogen. Bone deformities were observed in a baby whose mother had drunk anagyrine containing goats milk. The goat gave birth to stillborn kids with deformed legs. The goat had been foraging *L. latifolius*, containing high amounts of anagyrine. One of these North American lupins, *L. nootkatensis*, has been introduced in Iceland and is now widely spread in the country.

L. consentinii has caused numerous cases of hemimelia (incomplete development of distal part of the limbs) in lambs where mothers had grazed on this lupin. Also dwarfism in calves has been related to this lupin. The responsible alkaloid has not been identified, but the predominant alkaloid, multiflorine, is structurally related to the teratogen anagyrine. Multiflorine may occur in the edible lupin *L. albus* in amounts up to 18 % of the total alkaloids. It is not clear to what extent bitter varieties of the white lupins are grown and how much their seeds are used in foods in the Nordic countries.

It is not either clear whether the occasionally high amount of multiflorine in white lupins may be of concern. Further, the apparently high sensitivity to acute intoxication, especially in children should be better studied.

Bitter lupin varieties and lupin varieties with higher total contents of alkaloids than 200 mg/ kg are cultivated in Europe. So the possibility that lupin seeds with higher contents of total alkaloids than 200 mg/kg are marketed as 'sweet' varieties exist

Exposure to lupin alkaloids in the Nordic countries has been estimated based on highest recommended, highest but still relevant levels of alkaloids, maximum use of lupin seeds in bread, pasta and snacks and high intakes of these three food categories (see Section 5.4). The estimated exposures are accordingly for children (weighing 20 kg) 0.6 – 1.4 mg/kg b.w. and for adults (weighing 60 kg) 0.3 – 0.8 mg/kg b.w.

For comparison case reports indicate that acute intoxications of adults caused by lupin alkaloids from edible lupins may occur after intake of 25–46 mg lupin alkaloids per body weight and case stories concerning small children indicate that intake of 11–25 mg/kg b.w. may be lethal.

8. Recommendations

The scope of this report has been to focus on the toxicity of the alkaloids in white lupin (*Lupinus albus*) and in the narrow-leaved lupin (*Lupinus angustifolius*). Therefore, other inherent constituents with putative adverse effects in these lupins, e.g. allergens, isoflavones, erucic acid and high manganese content as well as contaminants, mycotoxins, especially the phomosins are not dealt with in details (see Section 4.3.3).

The following recommendations are given in order to better ensure the safe use of lupin seeds in Nordic foods:

Selection of lupins for food use

- Species and varieties / cultivars should be well-characterised
- Lupins with low alkaloid content should be preferred
- Lupins without or with negligible amounts of suspected teratogens, especially anagyrine, multiflorine and ammodendrine derivatives, should be preferred
- Methods to reduce lupin alkaloids (debittering processes) should be worked out under analytical control

Analysis / exposure

- Methods should be developed in Nordic countries for quantitative analysis of total lupin alkaloids and of selected alkaloids
- Lupin seeds from "sweet" lupin cultivars should be monitored for alkaloids (cross pollination from "bitter" lupins is possible)
- The total amounts of well characterised lupin seed products in specified foods/food categories should be given

Toxicity data

- Studies on absorption, distribution, metabolism and excretion are almost absent and should be performed
- It should be studied how relevant the results from the many rat studies are for humans
- If multiflorine (or other possibly teratogenic lupin alkaloids) occur in the lupin seeds used for foods, then these alkaloids should be studied for teratogenic effects

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