Bundesinstitut für Risikobewertung

DOI 10.17590/20170530-142504

Risk assessment of the occurrence of alkaloids in lupin seeds

BfR Opinion No 003/2017, 27 March 2017

For several years now, lupin flour has been used in bakery products, pasta, milk and soya substitutes, diet products, sauces and as additive to wheat flour. Lupin seeds, also known as lupini beans, are eaten as snacks in some European and North African countries.

Lupin seeds can contain toxicologically relevant bitter quinolizidine alkaloids which cause symptoms of poisoning in humans, affecting the nervous, circulatory and digestive systems. Typical symptoms of lupin alkaloid poisoning are dizziness, confusion, tachycardia, nausea, dry mouth, loss of motoric coordination and in high doses, cardiac arrest and respiratory paralysis.

The levels of quinolizidine alkaloids in lupin seeds vary depending on the botanical and geographical origin of the lupin variety from which theyderive. “Bitter lupins” produce seeds which have a bitter taste due to the higher levels of quinolizidine alkaloids they contain. Bitter lupin seeds are not suitable for human consumption without appropriate pre-treatment (“debittering”). Lupin varieties which produce seeds with low alkaloid levels and which have been obtained by specific cultivation are known as “sweet lupins” and they are suitable for human consumption even without debittering. It is usually very difficult for consumers to recognise whether the lupin seeds on offer are derived from sweet or bitter varieties. This has resulted in the past in isolated cases of poisoning in Germany caused by bitter lupin seeds.

The BfR recommends that the manufacturers of foods only market whole, uncrushed seeds, which may be consumed without the need for any debittering processes at home. These can be sweet lupin seeds with naturally low alkaloid levels, or bitter lupin seeds which have already been sufficiently debittered by the manufacturer. Where flour made from lupin seeds is to be sold to consumers, the manufacturers should ensure that it was made from lupin seeds which were low in alkaloids or sufficiently debittered before going on sale.

As a precaution, the BfR recommends that consumers who do not have sufficient knowledge of the subject avoid the consumption of lupin seeds which were not debittered by the manufacturer, as there is no certainty that the recommended debittering procedures result in a sufficient reduction in the levels of health-damaging alkaloids. If lupin seeds or products made from them have a bitter taste, this can be an indicator for the presence of undesired, health-damaging lupin alkaloids. The bitter-tasting water from soaking the lupin seeds for debittering should not be consumed under any circumstances or used for the preparation of foods.

BfR Risk Profile:
Occurrence of alkaloids in lupin seeds (opinion No 003/2017)

A	**Persons affected**	**High consumers of lupin seeds**				
B	**Likelihood of health impairment in high consumers of lupin seeds**	Practically impossible	Unlikely	**Possible**	Probable	Certain
C	**Severity of health impairments in high consumers of lupin seeds**	**The severity of impairments can vary**				
D	**Reliability of available data**	High: The most important data are available and free of contradiction		**Moderate: Some important data are missing**	Low: Numerous important data are missing or contradictory	
E	**Controllability by consumers**	No control necessary	**Controllable through precautionary measures**	Controllable through avoidance	Not controllable	

Boxes highlighted in dark blue indicate the properties of the risk assessed in this opinion
(more detailed information on this can be found in the text of BfR Opinion No 003/2017 of 27 March 2017).

Explanations

This risk profile is intended to visualise the risks outlined in the BfR Opinion. It should not be used for the comparison of risks. The risk profile should only be read in conjunction with the opinion.

Line E – Controllability by consumers
The information given in the line "Controllability by consumers" is of a purely descriptive character and should not be seen as a recommendation from the BfR. The BfR recommended a course of action in its opinion: Avoid bitter lupin seeds not debittered by the manufacturer.

FEDERAL INSTITUTE FOR RISK ASSESSMENT (BfR)

1 Object of the evaluation

The Federal Institute for Risk Assessment (BfR) has assessed the health risks of lupin seeds (lupini beans). This opinion relates to the risks associated with the alkaloid content of the seeds of lupin species and varieties with which deliberate consumption as a food has to be taken into account, namely *Lupinus albus L.* (white lupin), *Lupinus angustifolius* L. (blue lupin), *Lupinus luteus L.* (yellow lupin) and *Lupinus mutabilis* SWEET (Andean lupin) (cf. 3.1.1). The allergenic potential of the proteins that occur in lupin seeds (BfR 2011; EFSA 2005, 2014) as well as the possible contamination of lupin seeds with mycotoxins, in particular phomopsins and the possible related health risks (e.g. (Dirksen 2006; EFSA 2012b)) are not a subject of this opinion.

2 Result

- The main alkaloids contained in foods produced from the seeds of *L. albus L.* (white lupin), *L. angustifolius* L. (blue lupin), *L. luteus* L. (yellow lupin) and *L. mutabilis* are described as being lupanine, lupinine, and sparteine. These quinolizidine alkaloids produce typical symptoms of poisoning in humans affecting the nervous, circulatory and digestive systems.

- Only a few analytical methods have been described with which the various alkaloids can be quantified. None of these methods have been verified in a method validation study.

- The BfR does not currently have at its disposal any analytical data on the alkaloid levels of lupin seeds and products made from them which are traded as foods in Germany. It must be assumed, however, that bitter lupin seeds could be on the market the consumption of which, if insufficiently debittered, could possibly lead to acute lupin alkaloid poisoning, as has been described in multiple instances, for which anticholinergic syndrome is typical and which can be lethal if respiratory paralysis results.

- It has not been possible to estimate exposure up to now due to a lack of consumption data on foods in Germany that contain lupin seeds or a processed form thereof (e.g. lupin flour). To obtain current data, a representative “Consumer survey on the consumption of lupin seeds” commissioned by the BfR was conducted throughout Germany in January/February 2016, the results of which were used as the basis for the exposure observation.

- Where acute exposure estimation is concerned, the BfR assumes an alkaloid content of 200 mg/kg seeds. This results in the highest alkaloid intake values for foods containing lupin seeds in the categories “Lupini beans as a snack” (0.286 mg/kg body weight (bw) per day) and “Patties” (0.229 mg/kg bw/day). Intake through other foods from the remaining categories ranges from 0.003 to 0.057 mg/kg bw/day.

- When assessing the risks, the alkaloid intakes are compared with the pharmacological threshold dose of 0.2 mg/kg bw described for sparteine as the reference value. The margin of safety (MOS) to the threshold dose should take into account the uncertainty in the data base regarding toxicology and in particular possibel higher sensitivity of children, pregnant women and poor metabolisers and should amount to more than 1. The MOS of 1 that results for the first two food categories mentioned is regarded as inadequate. The MOS lies between 4 and 70 for the remaining categories.

- For industrial debittering of lupin seeds research results are described in the literature. These processes are not an object of this opinion.

- Where debittering processes at the consumer level are concerned, various methods are described, most of which involve boiling and soaking the seeds for several days while changing the water several times. However, no general recommendation can be made here by the BfR, as there are no systematic and validated tests of the quality of these methods, the success of which also depends on the variable initial lupin alkaloid content in the seeds. Thus, cases of poisoning have been attributed repeatedly to the insufficient debittering of bitter lupin seeds by consumers.

On the basis of the risk assessment conducted, the BfR has issued a number of detailed recommendations directed to producers, consumers, risk management and research institutions:

- The BfR recommends in particular that for direct preparation of meals or consumption (snacks), producers only bring whole, uncrushed lupin seeds into the market which can be eaten without the need for any further debittering processes. These can be sweet lupin seeds, which have naturally low alkaloid levels, or bitter lupin seeds

which have already been sufficiently debittered by the producer. Where lupin seed flour is to be sold to consumers, manufacturers should ensure that it is made from lupin seeds which are low in alkaloids or have been sufficiently debittered.

- The BfR recommends as a precaution that consumers avoid eating bitter lupin seeds which have not been debittered by the manufacturer as the recommended debittering procedures do not result with certainty in a sufficient reduction of the levels of health-damaging alkaloids. Consumers are made aware that if lupin seeds and products made from them have a bitter taste, this is an indication for the presence of lupin alkaloids which are undesired from a health perspective. The bitter-tasting water water from soaking the lupin seeds for debittering should not be consumed under any circumstances or used for the preparation of food.

3 Justification

3.1 Risk assessment

3.1.1 Agent

3.1.1.1 Botanical origin

The genus *Lupinus* L. comprises numerous species with a total of more than 500 taxa. It belongs to the *Leguminosae* family. Lupins are cultivated for green manuring, as decorative and fodder plants and for nutritional purposes. The seeds of *Lupinus albus* L. (white lupin) as well as *Lupinus angustifolius* L. (blue lupin) and *Lupinus luteus* L. (yellow lupin) are described as economically relevant for the manufacture of foods. Another cultured lupin of lesser significance is *Lupinus mutabilis* SWEET (Andean lupin), the seeds of which are also used for food production (Blaschek *et al.* 2016; Frohne & Pfänder 2004; Ternes *et al.* 2007). Only the protein-rich seeds of certain lupin varieties and the flours made from them have any significance as foods. Lupin seeds (lupini beans) are eaten as a snack in some European and North African countries and serve as a coffee substitute when roasted (Blaschek *et al.* 2016; Ternes *et al.* 2007). It is also reported that lupin flour has been used more and more in recent years in bakery products and pasta, dairy and soya substitute products, diet products, sauces and as an additive to wheat flour, partly after fermentation (EFSA 2005; Kohajdová *et al.* 2011). The seeds as well as the leaves of the lupin contain toxicologically relevant bitter quinolizidine alkaloids, levels of which vary greatly, depending on the botanical and geographical origin, as well as the soil composition and climate among other things (Gremigni *et al.* 2001; Jansen *et al.* 2009). It is described in the relevant literature that lupin seeds with higher levels of bitter quinolizidine alkaloids are not suitable for human consumption or as animal feed without pre-treatment (debittering) (Blaschek *et al.* 2016; Gessner & Orzechowski 1974). For example, for human nutrition the seeds are only used after days of debittering as is described for consumption of the the seeds of *L. albus* in southern Europe, and those of *L. mutabilis* by the Indios of Peru, (ANZFA 2001; Ternes *et al.* 2007).

Lupin varieties which produce seeds with low alkaloid levels and which are acquired through specific cultivation are known as “sweet lupins”, while those which have a bitter taste due to higher alkaloid levels are referred to as “bitter lupins” (ANZFA 2001; Gessner & Orzechowski 1974; Ternes *et al.* 2007). The alkaloid levels of sweet lupin seeds are given as ranging from 0.01 – 0.08 % (100 - 800 mg/kg) (Gessner & Orzechowski 1974). Other authors define the alkaloid levels in the seeds of sweet lupin varieties as $\leq$ 500 mg/kg dry mass and in those of bitter lupin varieties as $\geq$ 10,000 mg/kg dry mass. The Australia New Zealand Food Authority assumes average levels of 130 - 150 mg/kg alkaloids in sweet lupin seeds (ANZFA 2001).

Numerous high-yield, so-called sweet lupin varieties of *L. albus, L. angustifolius, L. luteus* and *L. mutabilis* are known to be cultivated all over the world in countries such as France, Spain, Portugal, Italy, Germany, Poland, Russia, South Africa, North and South America and Australia (Blaschek *et al.* 2016). Ternes et al. assert that numerous “alkaloid-free” varieties of *L. albus* and *L. luteus* exist (Ternes *et al.* 2007).

The following commercial varieties of sweet lupin are described:

- *L. albus* var. Lublanc, Lucky, Lutop, Multolupa, Amiga, WAT, Kiev, Ultra, Kaly, Estoril, Neutra, Golf, Achat, Ida, Ares and Llaima (Blaschek et al. 2016).
- *L. angustifolius* var. Unicrop, Wandoo, Chittick, Maresa (Blaschek et al. 2016).
- *L. luteus* var. Barpine, Topaz, Aurea, Barfin, Aga, Reda, Bornova, Borsaja, Borselfa, Schwako, Refusa, Baltyk (Blaschek et al. 2016).
- *L. mutabilis* var. INTI (Blaschek et al. 2016).

However, alkaloid-rich seed varieties of *L. albus, L. angustifolius, L. luteus* and *L. mutabilis* are also offered (*L. albus* var. Blanka, Kalina, Pop 1, Semu; *L. angustifolius* var. New Zealand Bitter Blue, Kubesa Turkus, Stevens, Mirela) (Blaschek *et al.* 2016).

Reports on the toxic effects and cases of poisoning among domestic animals in particular are available for certain lupin varieties that grow in the wild and which are not assessed individually here. Accordingly, the occurrence of deformities in calves (crooked calf syndrome) in the USA, Canada and Australia is associated with the intake of *L. sericus*, *L. caudatus, L. nootkatensis, L. laxiflorus* Lindl, *L. formosus* by the mother animals while grazing or through soilage. The neuropathogenic α-pyridone alkaloid anagyrine, as well as piperidine alkaloids (e.g. ammodendrine) which constitute the main alkaloids in some of these lupin varieties, are presumed to be the cause (Blaschek *et al.* 2016; Dirksen 2006). It was conspicuous that cases of hemimelia occurred frequently in lambs whose mothers had ingested *L. consentinii* while grazing, with a relationship to the level of multiflorin in this lupin species being presumed (Allen *et al.* 1983).

3.1.1.2 Composition of lupin seeds

Levels of storage proteins (35 - 55%), lipids (4 - 20%), oligosaccharides and secondary plant substances such as saponins and in particular alkaloids in lupin seeds vary depending on the species or variety. Typical of most lupin varieties is the occurrence of quinolizidine alkaloids such as lupanine[1], lupinine[2] and sparteine[3] (Blaschek *et al.* 2016; O'Neil 2006; Wood & Wrigglesworth 2008). Lupanine forms the main alkaloid in the seeds of *L. albus* and *L. angustifolius*. Lupinine dominates in the seeds of *L. luteus* and lupanine in those of *L. mutabilis,* each combined with sparteine as the main alkaloids (Blaschek *et al.* 2016; Boschin *et al.* 2008; de Cortes Sánchez *et al.* 2005; Kamel *et al.* 2015; Lee *et al.* 2007; Lubowicki *et al.* 2005; Reinhard *et al.* 2006; Resta *et al.* 2008b).

The BfR does not have any analysis data on the alkaloid levels of lupin seeds and products made from them which are marketed as foods in Germany. The following details are based on the results of literature research.

- Alkaloid levels in the seeds of *L. albus*:
  The major alkaloids of the seeds are lupanine (55 - 75% of total alkaloids), albine (6 - 15%), multiflorin (3 - 14%), 13-hydroxylupanine (4 to 12%), 13-angeloyloxylupanine (1 - 3%). Minor alkaloids of the seeds are ammodendrine, angustifolin, 5,6-dehydrolupanine, isoangustifoline, α-isolupanine, 17-oxolupanine, 11,12-seco-12,13-didehydro-multiflorin (previously N-methyl-albin), sparteine, tetrahydrocytisine, tetrahydrorhombifoline, various esters of 13-hydroxylupanine and 13-hydroxymultiflorin, 5,6-dehydromultiflorin, lupanine N-oxide and 13α-hydroxy-5-dehydromultiflorin (Blaschek et al. 2016). Seeds of sweet lupin varieties of *L. albus* show alkaloid levels of under 0.05% with some types reaching 50 micrograms per gram (µg/g) dry weight (0.005%). Bitter lupin seeds of the species *L. albus* grown in the wild can achieve up to 8% of the alkaloid levels (Blaschek et al. 2016).

- Alkaloid levels of the seeds of *L. angustifolius*:
  The major alkaloids of the seeds are lupanine (65 - 75% of total alkaloids), angustifolin (10 - 15%) and 13-hydroxylupanine (10 - 15%). Minor alkaloids of the seeds are isoangustifolin, isolupanine, 17-oxolupanine, sparteine and tetrahydrorhombifoline (Blaschek et al. 2016). Seeds of sweet lupin varieties of *L. angustifolius* show alkaloid levels of under 0.05%. With bitter lupin seeds of the species *L. angustifolius,* the alkaloid levels lie between 1.5 and 4% (Blaschek et al. 2016).

- Alkaloid levels of the seeds of *L. luteus*:
  The major alkaloids of the seeds are lupinine (60% of total alkaloids) and sparteine (30%). Minor alkaloids of the seeds are ammodendrine, feruloyl lupinine, β - isosparteine, lupanine, 17-oxosparteine and tetrahydrorhombifoline (Blaschek et al. 2016). Seeds of sweet lupin varieties of *L. luteus* have alkaloid levels of under 0.1%.

[1] (+) Lupanine was detected in *L. angustifolius* and racemic lupanine in *L. albus* (O'Neil 2006). The stereochemistry for lupanine (see Wood & Wrigglesworth 2008) is only indicated in the opinion if corresponding information was contained in the quoted literature.

[2] (-) Lupinine was detected in *L. luteus* and other lupin species (O'Neil 2006). The stereochemistry for lupinine (see Wood & Wrigglesworth 2008) is only indicated in the opinion if corresponding information was contained in the quoted literature.

[3] (-) Sparteine was detected in *L. luteus* and other lupin species (O'Neil 2006; Blaschek 2016). The stereochemistry for sparteine (see Wood & Wrigglesworth 2008) is only indicated in the opinion if corresponding information was contained in the quoted literature.

With bitter lupin seeds of the species *L. luteus,* the alkaloid levels lie between 0.5 and 2% (Blaschek et al. 2016).

- Alkaloid levels of the seeds of *L. mutabilis*:
  The major alkaloids of the seeds are lupanine (37 to 75%), 3β-hydroxylupanine (4 to 22%), 13α-hydroxylupanine (4 to 20%), sparteine (3 to 20%), tetrahydrorhombifoline (0.1 to 4%). Minor alkaloids of the seeds are ammodendrine, 13-angeloyloxylupanine, angustifolin, 11,12-dehydrosparteine, 3,13-dihydroxylupanine, α-isolupanine, multiflorin, 17-oxosparteine, 13-tigloyloxylupaninr and various esters of 13-hydroxylupanine, 3-hydroxylupanine and 3,13-dihydroxylupanine (Blaschek et al. 2016). Seeds of sweet lupin varieties of *L. mutabilis* show alkaloid levels of under 0.1% (minimum 0.001%) auf. With bitter lupin seeds of the species *L. mutabilis,* the alkaloid levels lie between 1 and 4% (Blaschek et al. 2016).

3.1.1.3 Influence of food preparation methods on the composition (debittering)

Research results of the industrial debittering of lupin seeds (Carvajal-Larenas *et al.* 2013; Ertas & Bilgicli 2014; Haddad *et al.* 2006) based on the water solubility of the quinolizidine alkaloids and which partly include fermentation processes (Jiménez-Martínez *et al.* 2007; Ortega-David & Rodriguez-Stouvenel 2013) are described in the literature. These methods are not a subject of this opinion.

Concerning household debittering processes, methods are also described most of which are based on a combination of boiling processes and soaking for several days with multiple changes of water (Bleitgen *et al.* 1979; Ertas & Bilgicli 2014; Fudiyansyah *et al.* 1995; Lowen *et al.* 1995; Pilegaard & Gry 2008; Smith 1987).

A typical debittering method described by various authors is based on the following instructions (Lowen *et al.* 1995; Smith 1987):

(1) Add 6 parts cold water to one part lupin seeds by volume and soak for 24 hours
(2) Pour off the soaking water and rinse the lupin seeds
(3) Add the same volume of water to the lupin seeds as described in 1, above, bring to boiling and simmer for 7 - 10 mins
(4) Pour off the hot water and rinse the lupin seeds
(5) Repeat steps 1 and 2 for 5 - 7 days changing the water three times a day until the lupin seeds no longer have a bitter taste.

Smith analysed the alkaloid decrease during the individual debittering stages and found out that alkaloids were transferred to the soaking water, even on the 6th day. The alkaloid transfer to the water was considerably higher on each of the two following days after boiling and soaking than it was in the other debittering stages.

Bleitgen et al. conducted experiments on the debittering of seeds of *L. mutabilis* var. H 1 and *L. albus* var. Astra using sensory tests (Bleitgen *et al.* 1979). They established that among other things, the swelling speed and swelling volume of the lupin seeds are of significance for debittering and that the boiling process enhances the purging of the alkaloids from the seeds. Based on their results, the authors recommended that the whole lupin seeds be boiled for half an hour and left under running water for three days when debittering at home. These results also showed that the bitter taste of lupin alkaloids in water could still be detected by sensors in the ppm range in the case of sparteine. There are severe differences in the degree of bitterness of lupin alkaloids. It decreased via d-lupanine perchlorate, lupinine, isolup-

anine towards hydroxylupanine. The ability of lupin seeds to swell had different characteristics depending on the variety. The swelling speed was slower with seeds of *L. albus* than with those of *L. mutabilis.*

It can be summarised , that there are no systematic and validated tests for the quality of household kitchen debittering methods. It is assumed that the success of debittering methods depends on various parameters including the variable initial content of lupin alkaloids in the seeds. Cases of poisoning were repeatedly attributed to insufficient debittering of bitter lupin seeds by consumers (cf. 3.1.2.3.2). This clearly indicates that the necessary debittering of bitter lupin seeds by consumers is a critical step from which their safety as a food depends. With the currently inadequate level of knowledge, no general recommendations can be made by the BfR regarding the debittering methods of bitter lupin seeds by consumers.

3.1.2 Hazard potential

Assessment-relevant data on the toxicology of seeds of *L. albus, L. angustifolius, L. luteus* and *L. mutabilis* after oral/peroral exposure are compiled below.

3.1.2.1 Statements on the alkaloid levels of lupin seeds made by national and international institutions

The Advisory Committee on Novel Food and Processes (ACNFP) in the United Kingdom published a health assessment of the seeds of *L. angustifolius (FSA 1996)* in 1996. The committee came to the conclusion **"**…*that seeds from L. angustifolius are safe for 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…*". This complied with the Maximum Permitted Concentrations (MPC)) already authorised in Australia (ANZFA 2001). The use of up to 10% lupin flour originating from the seeds of a low-alkaloid variety of *L. albus* (ARES) was accepted in France in 1998 provided that the alkaloid level does not exceed 200 mg/kg (Santé 1998).

The Australia New Zealand Food Authority (ANZFA) concluded in its health assessment (ANZFA 2001) that:

"The only data available on human chronic toxicity are the reports of traditional use of lupini beans in Europe, which indicate a daily dose of 0.35 mg/kg can be tolerated in adults without adverse effects. On the basis of this limited data, however, it is not appropriate to consider this dose level as the safe level for all individuals in the population. The only data available on the levels of alkaloids in lupini beans is anecdotal – there seems to be no published information available. Also, the information applies only to adults, not children, and it is likely that the adult population has developed a certain amount of tolerance to these alkaloids. The limited metabolism data available, however, suggests that the alkaloids are rapidly excreted unchanged which would reduce the likelihood of chronic toxicity. If a safety factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake (PTDI) for humans is 0.035 mg/kg/day or 35 µg/kg/day".[4]

[4] The dosage information relates to lupin alkaloids. The German version of the text is a self-translation of the English text which should be regarded as the official version.

ANZFA also provides information on the mean alkaloid levels of lupin seeds in the Australian market and developments in the cultivation of lupin varieties with low alkaloid levels (ANZFA 2001):

"Data indicates that the mean alkaloid content of marketable sweet lupin seed is on average 130 - 150 mg/kg."

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

Reference is made to a publication by the Nordic Council of Ministers which provides an overview of the occurrence of alkaloids in edible lupin seeds (Pilegaard & Gry 2008).

3.1.2.2 Data on toxicity

3.1.2.2.1 Mode of action

Lupin alkaloids showed *in vitro* an inhibitory effect on nicotinic and muscarinic acetylcholine receptors from pig's brain (Blaschek *et al.* 2016). The following half-maximal inhibitory concentrations (IC_{50} values) were determined at nicotinic acetylcholine receptors: lupanine 5 micromolar (µM), 3-hydroxylupanine 190 µM, albine 193 µM, tetrahydrorhombifoline 310 µM, sparteine 331 µM, lupinine, multiflorin over 500 µM (Blaschek *et al.* 2016). The following half-maximal inhibitory concentrations (IC_{50} values) were determined at muscarinic acetylcholine receptors: sparteine 21 µM, albine 33 µM, multiflorin 47 µM, 3-hydroxylupanine 74 µM, lupanine 114 µM, 13α -hydroxylupanine 140 µM, lupinine 190 µM (Blaschek *et al.* 2016).

Sparteine and lupanine showed very weak affinities to muscarinic acetylcholine receptors from rat's brain. The Ki (inhibition constant) amounted to 7,000 nanomolar (nM) for sparteine and 11,000 nM for lupanine; in comparison to this the Ki for atropine is 0.16 nM. The authors note that this result is not compatible with the pharmacodynamic activity observed *in vivo*. With a Ki of 400 nM and 500 nM respectively, sparteine and lupanine showed similar affinities to nicotinic acetylcholine receptors from rat's brain. These affinities were therefore relatively high and only roughly five times lower than with nicotine (Ki = 90 nM). Overall, considering also *in vivo* data on toxicology and pharmacology, the authors came to the conclusion that the pharmacological properties of sparteine and lupanine are similar although quantitative differences exist (see also 3.1.2.23.1 (a))(Yovo *et al.* 1984).

Sparteine blocks Na^+ channels and reduces the K^+ permeability of nerve and pancreas cells. It was also shown that sparteine and lupanin (300 µM respectively) have an inhibitory effect on sodium and potassium channels of isolated frog muscle cells (*Xenopus laevis*) (Blaschek *et al.* 2016).

Lupin alkaloids had a uterus-contracting effect in *vitro*. The effect of lupinine on an isolated rabbit uterus was only $^1/_5$ and that of lupanine dihydrochloride only $^1/_{15}$ as strong as sparteine sulphate (Gessner & Orzechowski 1974; Ligon 1941). Lupin alkaloids have an antiarrythmic effect on the isolated heart as they remove atrial and ventricular flutter by slowing down the

nervous conduction. The antiarrhythmic effect diminishes from sparteine via lupanine to 13-hydroxylupanine (Blaschek *et al.* 2016).

3.1.2.2.2 Toxicokinetics

Sparteine. In humans, 70% of the substance was absorbed from the gastrointestinal tract after the peroral administration of a dose of 200 mg of sparteine sulphate, with peak plasma levels being reached after 45 minutes. The elimination half-life is 117 mins. After i. v. administration of spartein sulphate, 34% of the substance is excreted with the urine as unchanged sparteine within 24 hours. Roughly 50% of the sparteine is bound to plasma protein. Sparteine is metabolised by *N*-oxidase. The first metabolite *N*-1 oxide is chemically unstable. 2-dehydro and 5-dehydro sparteine are created through dehydratation. These two metabolites cannot be formed by 5% of the population who, due to a genetic polymorphism, do not possess the cytochrom P450 isoenzyme CYP2D6. These nonmetabolisers, who have higher sparteine plasma levels than the normal population and who excrete more than 95% of the perorally administered dose as unchanged sparteine with the urine, are more susceptible to undesired sparteine effects (e.g. accomodation disturbances in the eye) than individuals with cytochrome P 450 metabolism (Aktories *et al.* 2009; Blaschek *et al.* 2006; Eichelbaum *et al.* 1979; Schomerus *et al.* 1978; Thies 1986).

Lupanine. Rats were given lupanine hydrochloride with their food. 70 - 80% of the ingested lupanine was excreted, 50 - 70% of it in urine and only 10 - 14% of it in faeces. Of the ingested lupanine, 30 - 40% was excreted as hydroxylised lupanine and roughly the same percentage unchanged with urine or faeces (Wittenburg & Nehring 1965). 10 mg of lupanine or 10 mg 13-hydroxylupanine were administered orally in a randomised test with a cross-over design with a two-week wash-out phase to 11 test persons, 7 of whom were classed as “extensive metabolisers“ (EM) and 4 as “poor metabolisers“ (PM) with regard to the presence of cytochrome P4502D6 (CYP2D6). The half-life times for lupanine were 6.2 ± 0.5 h (EM) and 6.5 ± 0.9 h (PM), with a recovery rate of the unchanged substance in urine within 72 hrs of 95.5 ± 6.0% and 89.9 ± 4.5% respectively. For 13-hydroxylupanine, the half-life values were 6.8 ± 1.0 h (EM) and 5.9 ± 1.6 h (PM), with a recovery rate of the unchanged substance in urine within 72 hrs of 100.5 ± 5.3% and 102.5 ± 4.8 % respectively. For the administered dose, there were no significant differences between the half-life times of the EM and PM individuals. In one of the EM and one of the PM individuals, 14% or 34%, respectively, of the administered 13-hydroxylupanine was dehydroxylised to lupanine. No adverse effects were detected in any of the test persons. Heart rate and blood pressure remained unaffected (Petterson *et al.* 1994).

3.1.2.2.3 Data on acute toxicity

Seeds of L. angustifolius and L. albus. In male Wistar rats, the oral LD_{50} of an extract of seeds of *L. angustifolius*, which contained 49% lupanine, 39% 13-hydroxylupanine, 10% angustifoline and 0.7% α-isolupanine, amounted to 2,279 mg/kg bw (animals fed before treatment) and 2,401 mg/kg bw (animals unfed 16 - 18 hours before treatment). 1 - 16 minutes after it was administered, the animals reacted with nerval symptoms such as tremor, followed by convulsions, cyanosis, collapse and death. Rats that survived the treatment showed no signs of clinical toxicity. The lowest lethal dose was 2,000 mg/kg bw (animals fed before treatment) and 2,600 mg/kg bw (animals unfed 16 - 18 hours before treatment) (Petterson *et al.* 1987). When extracts of seeds of *L. angustifolius* and *L. albus* were administered per feeding tube to male and female AoBoy/Iiw mice, a mean lethal dose (LD_{50}) of > 4 000 mg/kg bw was determined. The extracts of both lupin species contained 10% alkaloids. LD_{50} values

in the range of 750 – 4,000 mg/kg bw were determined for fractions produced with various solvents from the extracts of seeds of *L. angustifolius* (Stobiecki *et al.* 1993).

Lupanine and sparteine

The LD_{50} with peroral administration is given as 410 mg lupanine/kg bw for male EOPS Swiss mice. Tremors and tonic-clonic seizures occurred as symptoms. Death was caused by respiratory paralysis (Yovo *et al.* 1984). The LD_{50} with peroral administration is given as 220 mg sparteine/kg bw for male EOPS Swiss mice. Tremors and tonic-clonic seizures occurred as symptoms. Death was caused by respiratory paralysis (Yovo *et al.* 1984). Yovo et al. came to the conclusion that the pharmacological properties of sparteine and lupanine are similar although there are quantitative differences (see also 3.1.2.2(a)).

The LD_{50} with peroral administration lay at 1,664 mg lupanine/kg bw for unfed Wistar rats. The authors point out that thus, the LD_{50} of lupanine is lower than that of the extract of seeds of *L. angustifolius* examined under similar conditions, (see above). This indicates that the other components in the extract had a lower acute toxicity than lupanine or that its toxicity was weakened by interactions. The lowest lethal dose was 1,538 mg lupanine/kg bw (Petterson *et al.* 1987).

3.1.2.2.4 Data on subacute and subchronic toxicity

Seeds of L. angustifolius, L. albus and L. mutabilis

In a study conducted by Ballester et al. (Ballester *et al.* 1980), three groups each consisting of twelve 21 to 23-day-old Charles River rats were given feed for 112 days which a) consisted to 58.1% of *L. albus*[5] (alkaloid content of the sweet lupins: 0.051%; estimated alkaloid intake: 26.6 mg alkaloids/kg bw/day (EFSA 2012a)), b) consisted to 51.3% of *L. luteus*[6] (alkaloid content of the sweet lupins 0.091% estimated alkaloid intake: 42.3 mg alkaloids/kg bw/day (EFSA 2012a)) and c) did not have any added lupins. The treatment groups did not show any significant differences for any of the test parameters which included feed intake, body weight development and organ weights, as well as macroscopic and microscopic organ examinations. The “No observed adverse effect level” (NOAEL) is therefore the highest tested dose, equating to 26.6 mg *L. albus* alkaloids/kg bw/day or 42.3 mg *L. luteus* alkaloid/kg bw/day.

In a nine-month study, Wistar rats in groups each comprising 20 male and female animals were given feed which consisted to 51.8% of flour originating from seeds of *L. albus* (cultivar: Multolopa; lupin content of the lupin flour: 0.025%; estimated lupin intake: 11.7 mg lupanine/kg bw/day (EFSA 2012a); no details of the levels of other alkaloids). Compared to the control group, the treatment group showed significantly decreased relative liver weights. No other adverse effects were observed. A NOAEL for the entirety of existent alkaloids cannot be derived (Ballester *et al.* 1982).

In a study by Butler et al. (Butler *et al.* 1996), Sprague-Dawley rats in four groups each comprising 20 male and 20 female animals were given feed which, due to its content of flour originating from seeds of *L. angustifolius* contained roughly 2.9 – 6.6 mg lupin alkaloids/kg bw /day, on at least 90 to 98 days. One of the groups served as the control group. With the other

[5] No details of which plant parts were used, presumably the seeds
[6] No details of which plant parts were used, presumably the seeds

three groups (dose groups), 250, 1,050 or 5,050 mg lupin alkaloids/kg feed was added to the feed (equivalent to 22.5; 95 or 455 mg/kg bw/day (EFSA 2012a)). The relative liver weights of female animals in the highest dose group showed a dose-dependent increase compared to those of the control group. Altered foci of hepatocytes were seen in 5 females of the high dose group and one female of the low dose group, as well as in one male from the medium and low dose groups respectively. Haematological changes were determined on the 45th day of treatment. The number of erythrocytes and haematocrit values were reduced in animals of both sexes, as were the MCV (mean corpuscular volume) values of the male animals. With regard to these results, the authors arrived at the following conclusion: *"The changes in the haematological parameters in both sexes were small and the lack of a consistent and persistent dose-related response from the interim period to terminal kill suggests that these findings are not of biological significance."*

In a 90-day study conducted by Robbins et al. (Robbins *et al.* 1996) in which rats were given similar doses of lupin alkaloids in the form of an extract of seeds of *L. angustifolius* with their feed, as in the study by Butler et al., no haematological changes were seen. Sprague-Dawley rats in four groups, each comprising 20 males and 20 females, had been given feed containing 0, 100, 330, 1,000 or 5,000 lupin alkaloids/kg (according to the authors equivalent to about 0, 10, 30, 100 or 500 mg/kg bw/day). The authors described a body weight reductions in the two upper dose groups and derive a NOAEL of 30 mg/kg bw/day on this basis. As the body weight reductions could be caused solely by reduced feed intake as a result of the bitter taste of the lupin alkaloids, the authors are discussing whether a NOAEL of 100 mg/kg bw/day might be more adequate. Significantly higher relative liver weights were determined for both sexes in the highest dose group and for the males in the lowest dose group.

No adverse effects were established in a twelve-week feeding study conducted on Sprague-Dawley rats in which debittered seeds of *L. mutabilis* served as the sole protein source (Schoeneberger *et al.* 1987).

The findings of the subchronic studies are inconsistent and sometimes contradictory as regards the observed liver changes (reduction or increase of relative liver weights, no confirmation of the induction of liver foci observed in one study by the results of other studies) and haematological changes. They are not regarded as a suitable basis for a risk assessment.

3.1.2.2.5 Data on chronic toxicity

Long-term feeding studies were conducted on animals – especially rodents – with the main goal of examining the nutritional benefits of seeds of *L. angustifolius* and *L. albus* (Grant *et al.* 1995; Grant *et al.* 1993; Jecsai *et al.* 1986; Rahman 2000). In many cases, the alkaloid content of the administered seeds was not given. With regard to the study design, these studies are not suitable for evaluating possible chronic toxicity or carcinogenicity.

3.1.2.2.6 Data on developmental and reproductive toxicity

Wistar rats in the F_1 and F_2 generations (groups each comprising 20 males and 20 females) originating from the parent generation F_0 of the subchronic study outlined above (Ballester *et al.* 1982) were given feed which consisted to 51.8% of flour derived from seeds of *L. albus* (cultivar: Multolopa; lupin content of the lupin flour: 0.025%; estimated lupin intake: 11.7 mg lupanine/kg bw/day (EFSA 2012a); no details of the levels of other alkaloids). A significant reduction of the relative liver weights was described as the only difference between the treated males and females and the control animals (Ballester *et al.* 1984).

A need for research is seen with regard to the possible developmental toxic effect of certain lupin alkaloids, such as ammodendrine and multiflorin and their derivates which occur mainly in wild lupins but also in traces in lupin seeds used for human nutrition (cf. 3.1.1. (a)).

3.1.2.2.7 Data on genotoxicity

It is mentioned in an overview article (Petterson 1998) that the testing of an alkaloid preparation of *L. angustifolius* on *Salmonella typhimurium* with and without metabolic activation had a negative outcome (original test reports and more detailed information not available).

3.1.2.3 Human data

3.1.2.3.1 Pharmacological and toxicological effects

Lupin seeds. The information is to be found in standard literature that the symptoms of lupin seed poisoning set in 20 mins after consumption of the seeds and that the maximum alkaloid effect occurs after 4 - 5 hours. Less severe cases of poisoning express themselves through accomodation disorders, vomiting, stomach ache, diarrhoea and cardiac complaints. In severe cases of poisoning conspicuous tiredness accompanied by curare-like paralysis and convulsions after 2 - 3 hours have been observed. It has been described that death can occur through choking while the heart is still beatingadequately. However, the paralysis can also be accompanied by tachyarrhythmia and cardiac arrest (Blaschek *et al.* 2006; Forrester 2006; Schmidlin-Mészáros 1973). More details can be taken from the case descriptions in the following chapters.

Sparteine

Sparteine has been used clinically in the form of sparteine sulphate since 1873 for the treatment of cardiac arrhythmia, because it inhibits excitiation and transmission at the heart in a manner similar to quinidine. There have been reports of antifibrillatory properties in an oral dose of 20 mg of sparteine sulphate (equivalent to 0.29 mg/kg bw assuming a body weight

of 70 kg). The therapeutic dose of sparteine sulphate was given with 4 mg/kg bw at maximum (Blaschek *et al.* 2006; Thies 1986). Sparteine sulphate was used in the late 1960s in Germany in higher individual doses of 100 mg/tablet or ampule (trade name: Depasan^{R}) as a Class I antiarrhythmic (sodium channel blocker) in the treatment of tachycardial arrhythmia. Experience showed, however, that the 100 mg dose (equivalent to 1.4 mg/kg bw assuming a body weight of 70 kg) still represented a lower threshold dose and that reliable treatment success was only achieved with an oral dose of 150 - 200 mg (equivalent to 2.1 to 2.9 mg/kg bw assuming a body weight of 70 kg). At this higher dose, however, nonmetabolisers are described as a new risk group. The recommendation to use higher doses was therefore only made with regard to patients with a normal metabolisation capacity (Thies 1986).

Central nervous disorders, negative inotropy (weakly pronounced in the therapeutic range) bradycardia, hypertonia, changes in haemogram and liver enzymes have been indicated as undesired effects. Use during pregnancy is contraindicated (Aktories *et al.* 2009; Blaschek *et al.* 2006; Thies 1986).

Sparteine also shows an oxytocic effect, i.e. it produces a stimulation of the muscles in the virginal, puerperal and gravid uterus. Sparteine produces rhythmic uterus contractions during the expulsive phase of childbirth (Thies 1986). Injected intramuscularly (i.m.), sparteine sulphate was therefore used as a reliable medication to intensify contractions with individual doses of 100 to 150 mg (equivalent to 1.1 to 2.1 mg/kg bw assuming a body weight of 70 kg) being administered (Gessner & Orzechowski 1974). Sparteine sulphate was also used in the USA for several years to induce labour (Newton *et al.* 1966; Schulman & Ledger 1965) until its use as “a potent oxytocic“ was banned by the FDA due to the unforeseeable occurrence of hypertonic uterine contractions which could not be influenced (FDA 1979; Thies 1986). It is discussed in the literature whether the women who reacted with tetania uteri could have been “nonmetabolisers” (Thies 1986). The effect of sparteine sulphate on the uterus when administered orally was examined by Dipont (Dipont 1971). Sparteine sulphate was administered in doses of 100 - 150 mg in the first phase of childbirth with the dose being repeated every hour if necessary. Through the treatment, a statistically significant shortening of the first phase of childbirth was achieved compared to the control group. The average administered dose of sparteine sulphate was 402.5 mg (Dipont 1971).

Reference is made in the standard literature to cases of poisoning through medications containing sparteine and it was assumed that this was observed in particular in individuals who were not capable of metabolising sparteine (see 3.1.2.2) (Aktories *et al.* 2009). Some cases of sparteine poisoninghad a fatal outcome. Sparteine peripherally produces a curare-like effect which initially leads to respiratory arrest through paralysis of the phrenic nerve endings. Larger doses paralyse the respiratory centre (Schmidt 1961). Sparteine is said to result in respiratory paralysis in humans from 40 mg/kg bw and in cardiac arrest from 90 mg/kg bw onwards (Blaschek *et al.* 2006; Thies 1986). There is a report in section (c) on the death of a child after the intake of 30 mg of sparteine/kg bw. The symptoms of poisoning are drowsiness, dizziness, headache, sweating outbreaks, mydriasis and myasthenia. Bradycardia sets in with high doses. Death results in consequence of respiratory paralysis (Aktories *et al.* 2009).

3.1.2.3.2 Case data

Two related medical reports (in line with Art. 16e (2) of the German Chemicals Law) from the year 1997 were found in the BfR case database under the keyword “Lupin”:

- A 4-year-old girl allegedly ingested a maximum of 22 lupin seeds orally in kindergarten and was monitored during a stay in hospital. No symptoms occurred.

- A 3-year-old girl allegedly ingested a maximum of 22 lupin seeds orally in kindergarten and was monitored during a stay in hospital. No symptoms occurred.

The BfR also approached 8 German poison information centres (GIZs) with a questionnaire in order to record human exposure to lupins and the resultant cases of illness. The evaluation of the survey, in which all 8 GIZs participated, showed that in the period from 2010 to 2015, 130 inquiries were made on the topic, 124 of them relating to oral exposure. The aggregate survey results are shown in Annex 1. Of the 124 inquiries about oral exposure, 106 (85.5%) concerned the intake of plant parts or foods. Of these, 97 (91.5%) related to exposure to plant parts (seeds, pods, sap, blossoms) and 6 (5.7%) to cases of exposure to foods (tea/infusion made from plant leaves, lupin seed flour, milk shakes). The noxa was not known for 3 (2.8%) of the oral exposures.

The degree of severity is known for 92 (73.6%) of the inquiries about oral human exposure, 70 of which (76%) were asymptomatic and 22 (24%) minor cases. After drinking an infusion prepared from “Andean lupins”, one woman experienced mydriasis, tachycardia, nausea and slurring which were assessed by the reporting GIZ as mild symptoms (in line with the Poisoning Severity Score (Persson *et al.* 1998)). This is the only case for which (mild) anticholinergic symptoms were reported. As there are no details as to which parts of the Andean lupins (*Lupinus mutabilis*) were used to make the infusion, the findings are regarded as not conclusive for the issues dealt with here.

The GIZ-Nord (Göttingen) reports in detail about the proportion of exposures involving lupin seeds and lupin pods in 28 out of 34 inquiries (82.5%). Of these, 2 persons (7%) had mild symptoms (a 9-year-old girl had consumed 5 seeds and a 2-year-old boy had eaten an unknown quantity of lupin pods). The GIZ-Freiburg reports mild symptoms in 2 adults after drinking an infusion made from the leaves and/or after smoking the leaves.

More details from the GIZ on selected individual instances are given in Annex 2 with regard to the noxa and symptoms.

Overall, it can be summarised that the evaluations made here is based on cases of poisoning after the oral intake of lupin seeds, pods or flour. The data available to the BfR do not permit a statement as to whether the observed sysmptoms have a causal relationship to the consumption of lupin seeds, pods or flour. Neither the BfR nor the German poison information centres have received any reports of moderate or severe cases of poisoning after the consumption of lupin seeds, pods or flour. After consumption of this kind, the state of health was merely described as “asymptomatic” or the observed symptoms as mild.

A further selection of published cases of poisoning is presented below with main focus placed on reports which describe a relationship between the ingested alkaloid dose and observed poisoning symptoms, especially in the low dose range. Several cases with typical symptoms are also presented. The botanical origin of the lupin seeds remains unclear in several case reports. *L. albus* and *L. flavus* are named as species whose seeds have led to cases of poisoning (Awada *et al.* 2011; Schmidlin-Mészáros 1973).

It becomes clear from the available literature that cases of poisoning can be frequently attributed to inadequate debittering of bitter lupin seeds by consumers (Awada *et al.* 2011; Daverio *et al.* 2014; Jamali 2011; Kurzbaum *et al.* 2008; Litkey & Dailey 2007; Lowen *et al.*

1995; Smith 1987). In one case, the poisoning was even caused by the consumption of the soaking water used for debittering (Luque Marquez *et al.* 1991).

Seeds of bitter lupins

Three lethal cases of the poisoning of children after the intake of lupin seeds are described. The poisoning pattern of a ten year-old boy (bw approx 30 kg) who ingested an unknown number of strongly bitter seeds of *L. albus* (approx. 25 - 30 g, presumed alkaloid dose: approx. 17 to 20 mg/kg bw) is typical in that he developed gastric symptoms, mydriasis, clonic convulsions, dyspnoea and cyanosis. He died under convulsions after 3 hours. There are also reports of the death of a one and a half-year-old child (bw approx. 9 kg) after the intake of six seeds of L. *albus* (presumed alkaloid dose: approx. 11 - 22 mg/kg bw). A 17 month-old child (bw approx 8 kg) died under signs of paralysis after it had eaten several lupin seeds of unknown botanical origin (5 – 10 g) containing over 1% sparteine and lupinine. The alkaloid dose ingested with the seeds was estimated at 12 - 25 mg/kg bw (Schmidlin-Mészáros 1973; Schmidt 1961).

In a grown woman (bw approx. 55 kg), the consumption of 70 - 80 g of uncooked dry seeds of *L. albus* (presumed alkaloid dose: approx. 25 - 29 mg/kg bw) which had been soaked in water led to poisoning with severe stomach pains, vomiting, pronounced visual impairments, severe mydriasis, dry throat, non-detectable pulse, excitement and dispnoea (Schmidlin-Mészáros 1973). After an intake of 100 - 150 g of uncooked dry lupin seeds (presumed dose: approx. 31 - 46 mg total alkaloids/kg bw, of which approx. 23 - 34 mg lupanine/kg Kbw), an adult male (bw approx. 65 kg) showed signs of poisoning with nausea, mydriasis and temporary coma (Schmidlin-Mészáros 1973).

Sparteine

A child of just under two and a half years of age died 3 hours after ingesting 413 mg of sparteine (in the form of a proprietary medication) equivalent to roughly 30 mg of sparteine/kg bw. The child first becam conspicuously tired before suffering increasing respiratory paralysis with simultaneous intense pulse acceleration. Tonic seizures then occurred before death set in (Schmidt 1961).

From the case data presented it can be concluded that lupin seed poisoning does not occur frequently. It does pose a serious threat to those affected, however, especially small children with whom cases of poisoning with a deadly outcome have been described. Cases of poisoning have been attributed repeatedly to inadequate debittering of bitter lupin seeds in the kitchen (Awada *et al.* 2011; Daverio *et al.* 2014; Jamali 2011; Kurzbaum *et al.* 2008; Litkey & Dailey 2007; Lowen *et al.* 1995; Smith 1987). There are no indications that the consumption of industrially produced foods made from lupin seeds has been associated with poisoning.

3.1.2.4 Point of departure for the characterisation of the hazard potential

The data situation on the effects and in particular on the dose-response relationships of the individual alkaloids and their interactions is sparse and not sufficient for the derivation of health-based guidance values, (HBGV) which, *if complied would ensure that the occurrence of acute or chronic health impairments would no longer have to be expected..* Where acute toxicity is concerned, there is a lack of data in particular on the No Effect Level of the quinolizidine alkaloids regarding the induction of uterine contractions after oral exposure. Where longer term exposure is concerned, there is a shortage of information on the chronic, developmental toxic, reproductive toxic and genotoxic effect.

If the human data from cases of poisoning is compared with the findings of experiments with animals on acute and longer-term toxicity, it becomes clear that humans quite obviously react more sensitively to quinolizidine alkaloids than laboratory rodents do. Thus, for example, the LD_{50} for male EOPS Swiss mice with peroral administration lies at 220 mg/kg bw for sparteine, whereas sparteine proved deadly for a small child at doses of about 30 mg/kg bw.

For this reason, when establishing a basis for estimating which intake quantities of quinolizidine alkaloids are tolerable with regard to the avoidance of toxic effects, human data are taken into consideration with a higher priority than findings from animal experiments . Human data are available from records of previous pharmaceutical uses of sparteine which, according to existing knowledge, is similar to the two other main alkaloids lupanine and lupinine in its pharmacological effect as an anticholinergic substance in terms of quality but which has a higher potency in terms of quantity. Accordingly, it showed greater acute toxicity in animal experiments (cf. 3.1.2.3.1a) and more activity *in vitro* on isolated hearts and an isolated uterus (cf. 3.1.2.2a). A conservative assessment approach is guaranteed this way, with sparteine being described as a component in the seeds of all four cultivated lupin varieties *L. luteus, L. mutabilis, L. albus a*nd *L. angustifolius*, but only as the main alkaloid in the first two.

20 mg of sparteine sulphate (equivalent to 0.29 mg sparteine sulphate /kg bw assuming a body weight of 70 kg) is given as the lowest pharmacologically effective (antifibrillatory) individual oral dose for the treatment of cardiac arrhythmia. Later experience shows, however, that a certain treatment success was only achieved with oral doses of 150 - 200 mg (equivalent to 2.1 – 2.9 mg/kg bw assuming a body weight of 70 kg) but that this dose also poses the risk of undesired effects for nonmetabolisers (Blaschek *et al.* 2006; Thies 1986).

For this reason, a dose of 0.29 mg sparteine sulphate/kg bw, the equivalent of 0.20 mg sparteine/kg bw, which was described in older literature as the lowest pharmacologically effective dose and which is regarded as the threshold dose, was selected as the point of departure for the characterisation of the acute hazard potential. In the risk assessment that has to be conducted, this dose is used as a reference value for a Margin of Safety (MOS) approach. In doing so, the MOS should take into account the uncertainty in the data base and in particular the possible higher sensitivity of children, pregnant women and poor metabolisers.

A toxicological reference value which could be used to estimate the risks of longer term exposure cannot be derived on the current level of knowledge.

3.1.3 Analytics

Lupins and lupin products have been included in the group of allergenics which must appear in the labelling of foodstuffs in Directive 2006/142/EC amending Annex III a of Directive 2000/13/EC and methods for validating this labelling obligation are available and accredited in DIN EN ISO 17025. These methods involve above all the detection of lupin proteins by means of ELISA and DNA by means of PCR.

The secondary plant ingredients contained in lupins belong mainly to the group of quinolizidine alkaloids. The main representatives of the detected quinolizidine alkaloids are: lupinine, lupanine, sparteine, α-isolupanine, 13-hydroxylupanine, angustifoline, albine, 13α-tigloyloxylupanine, multiflorin, tetrahydrorhombifoline.

Other alkaloids that occur in lupins include those of the piperidine type (ammodendrine).

The profiles of the alkaloids contained in lupins depend among other things on the variety, the plant part examined, the stage of development, geographic origin and stress factors (Boschin *et al.* 2008; Chludil *et al.* 2009).

Most of the methods described in the literature serve to identify the various alkaloids in the various lupin varieties. The identification is made above all via the mass spectra prepared with GC-MS and a comparison with a mass spectral library database. The percentage of the individual alkaloids in the samples can be estimated by means of the mass spectra. This semiquantitative determination was conducted above all for seeds and various plants and plant parts (Boschin *et al.* 2008; Chludil *et al.* 2009; Resta *et al.* 2008a; Wink *et al.* 1995).

Few methods for quantifiying the alkaloids in lupins and foods made from lupins are described in the literature. These methods are mainly based on GC-MS (Boschin *et al.* 2008; Reinhard *et al.* 2006; Resta *et al.* 2008a). A capillary electrophoresis MS method for foods is also described (Ganzera *et al.* 2010). An LC-MS/MS multimethod, which detects the alkaloids sparteine and lupanine among others in the blood, was developed for the examination of possible poisoning of humans by plants (Carlier *et al.* 2015). The principle of purification is similar in all methods and for the various matrices. After acidic extraction, the extracts are alkalised and further purified by means of liquid-liquid or solid phase extraction. Analysis then follows using the corresponding methods.

The levels in lupin seeds and foods made from lupin seeds, such as flour, bakery products, tofu and coffee, were examined. The quantification of the various alkaloids is problematic as only a few of the substances are available as standards and no isotope-marked internal standards are available either. In several of the described methods, various alkaloids were isolated from the plants, purified and then used as standards for quantification.

The detection limits and limits of quantitation of the methods listed in the literature differ widely, depending on the area of application, method used and the analyte. They extend from a detection limit of 0.1 micrograms/litre (µg/l) for lupanine in blood and analysis with LC-MS/MS up to 30 mg/kg for sparteine in lupin seeds and analysis with capillary electrophoresis. The achieved recoveries are in the range of 90% to 110% for all methods with a variation coefficient of under 10% as the yardstick for repeatability. Several of the methods described have been validated by the BfR but none of them have been validated in a mezhod validation study with several laboratories.

Synopsis:

- Few methods are described which quantify the various alkaloids.
- The availability of the standard substances is limited and isotope-marked standards are not yet available according to research conducted by the BfR. This limits the reliability of quantification, especially with GC-MS methods.
- Only a few methods have been validated internally by the laboratory and none of them have been validated in a method validation study. None of the methods have yet been accredited in line with DIN EN ISO 17025 either, according to research conducted by the BfR.

3.1.4 Exposure

3.1.4.1 Consumption of foods with lupin seeds

To date, there has been a lack of consumption data on foods in Germany that contain lupin seeds or a processed form of lupin seeds (e.g. lupin flour). In order to obtain the latest data, a nationwide, representative “Consumer survey on the consumption of lupin seeds” commissioned by the BfR was conducted in January/February 2016. Of the 2,022 study participants, 1,021 were men and 1,001 women. The survey was conducted among the population resident in Germany aged 14 years and over.

An analysis of the range of foods containing lupin seeds was conducted prior to the survey with the help of MINTEL database (MINTEL 2016). Among other things, this database contains information on foods and their ingredients as listed on the packaging. Research on products with which the word “lupin*” is to be found in the list of ingredients showed that in the years 2005 – 2015, a total of 278 foods of this kind that included lupin seeds/flour in the list of ingredients were put on the market in Germany. These foods are to be found mainly in the categories “Sweet Pastries/Biscuits”, “Bread and Bakery Products” and “Cakes & Confectionery”. The categories for the “Survey on the consumption of lupin seeds” were formed under consideration of this information. The results of the survey show how frequently foods with lupin seeds from the following categories were consumed in the last 12 months (Table 1).

Table 1: Food categories in which foods with lupin seeds are consumed and frequency of consumption of foods containing lupin seeds in the individual categories

Food Category	n (% of 2,022)	Frequencies in %				
		Several times a week	Once a week	Once a fortnight	Roughly once a month	Less often than once a month but at least once in the last year
Lupini beans as a snack	51 (2.5)	9.8	3.9	7.8	13.7	64.7
Confectionery	28 (1.4)	3.4	6.9	0.0	20.7	69.0
Patties/meat substitutes	51 (2.5)	0.0	12.0	4.0	22.0	62.0
Muesli/breakfast cereals	23 (1.1)	16.0	24.0	8.0	16.0	36.0
Biscuits	42 (2.1)	0.0	16.7	7.1	9.5	66.7
Cakes	42 (2.1)	0.0	16.7	7.1	9.5	66.7
Bread	61 (3.0)	6.6	14.8	6.6	19.7	52.5
Spreads	21 (1.0)	4.5	9.1	4.5	18.2	63.6
Smoothies/shakes	15 (0.7)	0.0	14.3	7.1	14.3	64.3
Ice-cream/dessert on a lupin basis	21 (1.0)	9.5	9.5	0.0	19.0	61.9
Dietary supplements (DS)	22 (1.1)	13.0	8.7	21.7	26.1	30.4

n – Number of respondents who have eaten foods with lupin seeds in this category at least once in the last 12 months

In addition to the respondents listed in Table 1, 75 participants (as the total of all categories) stated that they “had not consumed a food with lupin seeds from any of the available categories in the last twelve months” and 29 were not able to give an answer. The categories “Not in the last 12 months” and “No answer” are not included in the table because only the last 12 months are taken into consideration in this observation.

Of the study participants who were aware of the edibility of lupin seeds (19% of all respondents), 46% had consciously eaten an industrially produced or self-prepared food containing lupin seeds from one of the listed food categories at least once. Table 1 shows the food categories that were asked about and the number of study participants who have already consumed foods with lupin seeds from the individual categories. It becomes clear that the frequencies with which products from the food categories mentioned are consumed mostly lie in the range “Less often than once a month but at least once in the last year”. Accordingly, foods containing lupin seeds are currently only consumed occasionally.

The results of the survey confirm the assumption that the percentage of respondents who have consciously eaten food with lupin seeds is small (9.2% of respondents). 6.7% of the respondents had eaten a food with lupin seeds at least once in the last 12 months. The percentage of those who specifically buy (and prepare) lupin seeds for this purpose and do not purchase a processed product or lupin flour is very small (1.2% of respondents).

The question regarding the purchasing source of lupin seeds themselves, which permitted multiple answers, showed that 74% buy them in an organic shop, 48% in a health food store and 43% in a grocery shop/supermarket, while 18% collect or harvest them by themselves and 11% buy them in a grocery store in which mainly Turkish, Arab, Lebanese, Mediterranean or Asian foods are sold (Fig.1). Only those persons were asked who prepare foods with lupin seeds by themselves and use lupin seeds to do so and not those using lupin flour and similar products (n = 7).

Of the 397 respondents who knew about lupin seeds, only 21% knew that a distinction is made between sweet and bitter lupin seeds.

Figure 1: Place of purchase of lupin seeds

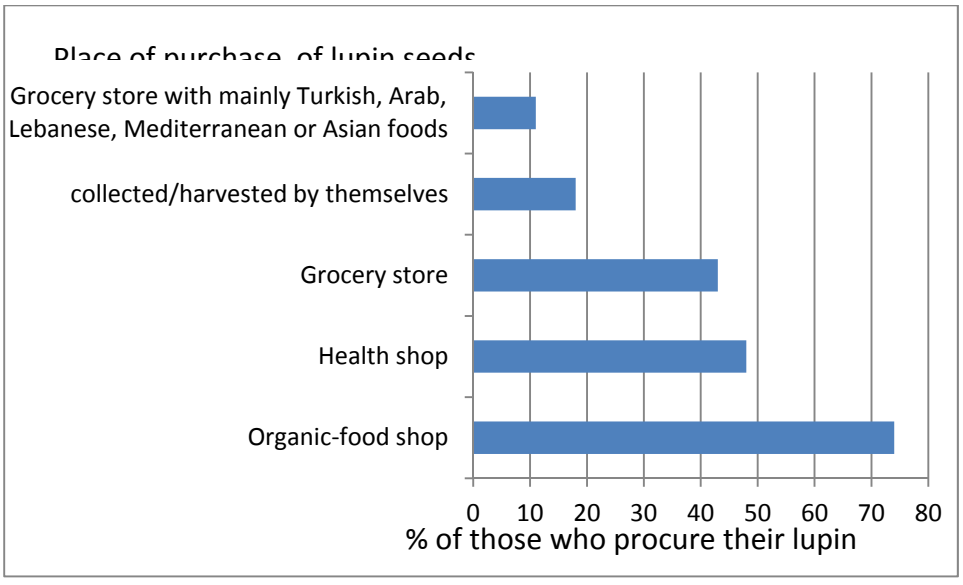
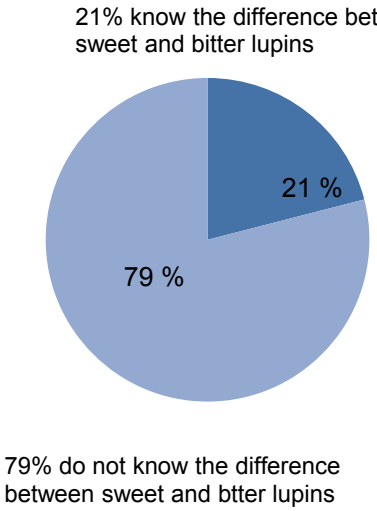


Figure 2: Level of knowledge among respondents regarding the distinction between sweet and bitter lupins



3.1.4.2 Content data

Reference is made to the following content data with regard to exposure estimation (see also 3.1.1 and 3.1.2.1):
According to the Australia New Zealand Food Authority, the average alkaloid content in sweet lupin seeds is 130 - 150 mg/kg lupin seeds (ANZFA 2001). The food authorities of Australia, New Zealand, the UK and France have determined a maximum value of 200 mg/kg for the alkaloid content in lupin seeds an lupin products made from them (Resta *et al.* 2008a) (cf. 3.1.2.1).

Levels of up to 500 mg/kg are found in bitter lupin seeds (Pilegaard & Gry 2008). A Swedish sample which triggered an alert in the European Rapid Alert System for Food and Feed (RASFF report) was found to have a lupanine level of 20,000 mg/kg seed.

An Italian research team headed by Donatella Resta examined commercial lupin-based products among others and only found alkaloid levels below 60 mg/kg in the foods (no details of the percentage of lupin seeds in the individual foods) (Resta *et al.* 2008a).

3.1.4.3 Calculation of chronic alkaloid intake via lupin-based foods

The calculation of alkaloid intake for the adult population was made on the basis of the variable portion size, lupin percentage in the food, frequency of consumption and alkaloid content of the food. The German Food Code and Nutrient Database (BLS), and where available, information provided by the manufacturer and packaging information, were used to calculate the percentage of lupin seeds in foods as well as the portion sizes. Where portion sizes and lupin seed percentages differed within a food category, a conservative approach was taken and the larger number was used for exposure estimation. Table 2 provides an overview.

Table 2: Portion sizes and percentage of lupin seeds in foods for the selected food categories

Foods with lupins	Researched		Source		Used for exposure estimation:	
	Portion size	% lupins in food	of portion size	of lupin percentage	portion size in g	percentage of lupins in food
Lupini beans as a snack	100 g	100	BLS	Manufacturer's data	100	100
Confectionery	50 g	2	BLS	Manufacturer's data	50	2
Patties	200 g	10 - 40	BLS	Manufacturer's data (alberts)	200	40
Muesli	30 - 40 g and 80 - 85 g	10 - 18	Packaging info Portion tub to go	Manufacturer's data (alvito Basenzeit, Rapunzel Sportlerbrei)	100	18
Biscuits	50 g	2	BLS	Manufacturer's data (alnavit)	50	2
Cakes	150 g (1 pc.)	5 - 10	BLS	Manufacturer's data	150	10
Bread	45 - 60 g 2 slices (1 roll)	3 - 9	BLS	Manufacturer's data Mintel DB	60	10
Spreads	30 g	30	BLS	Manufacturer's data (Zwergenwiese)	30	30
Smoothies/ shakes	250 ml liquid + 20 g lupin seed powder	8	Packaging info, manufacturer's data (Raab Vitalfoods)	Manufacturer's data (Raab Vitalfoods)	250	8
Ice-cream/ dessert	Dessert: 150 g (1 ball of ice-cream = 60 g)	1,5-2,7	Packaging info	Manufacturer's data	150	3
DS	20 g and 8 - 12 g (equates to 2 - 3 teaspoons of powder)	100	Manufacturer's data (Raab Vitalfoods , effective nature)	Manufacturer's data	20	100

The frequencies of consumption of the various foods containing lupin seeds from the population survey were converted into an intake probability per day for exposure estimation (Table 3). The assumption was made here that "Several times a week" equates to consumption on five days. The factor for "Less often than once a month but at least once a year" also assumes consumption on six days.

Table 3:Factors for frequency

Details of frequency	Factor for exposure estimation
Several times a week	5 / 7 (260/365)
Roughly once a week	1 / 7 (52/365)
Roughly once a fortnight	1 / 14 (26/365)
Roughly once a month	1 / 30 (12/365)
Less often than once a month but at least once a year	6 / 365

Exposure estimation for chronic alkaloid intake is made on the basis of consumers and using the portion sizes, percentage of lupin seeds in the food, consumption frequencies and alkaloid content of the lupin seeds in the food of 60 mg/kg.

Table 4: Chronic alkaloid intake in the individual food categories

Food category	Consumers in % (n)	Median in mg/kg bw/day
Lupini beans as a snack	2.5 (51)	0.00705
Confectionery	1.4 (28)	0.00007
Patties	2.5 (51)	0.00564
Muesli	1.1 (23)	0.00221
Biscuits	2.1 (42)	0.00007
Cakes	2.1 (42)	0.00106
Bread	3.0 (61)	0.00042
Spreads	1.0 (21)	0.00063
Shakes	0.7 (15)	0.00141
Dessert/ ice-cream	1.0 (21)	0.00032
DS	1.1 (22)	0.00229

As the numbers of consumers of the individual foods are very different, the calculation of total intake over all food categories and all consumers would lead to a distorted picture. For this reason, total intake was determined for the consumers in the two food groups with the highest alkaloid intake. Table 4 shows that alkaloid intake is highest in the food categories “Lupini beans as a snack” and “Patties” and that the number of consumers is also higher here than in the other categories (except Bread). Consumers of foods from these two categories form the calculation basis of the following exposure estimation. Chronic overall intake of lupin alkaloids for consumers of “Lupini beans as a snack” and “Patties” is identical and amounts to 0.013 mg/kg bw/day (median). As only 6.7% of the 2,022 respondents have consumed a lupin-based food in the last 12 months, more than 90% of the overall population has been covered by the observation of the median of consumers and consequently, no additional 95^{th} percentile is given.

3.1.4.4 Calculation of acute alkaloid intake via lupin-based foods

Acute exposure was calculated on the basis of the portion sizes, percentage of lupin seeds in the food and under the assumption of an alkaloid content of 200 mg/kg lupin seeds in the food (Table 5). A standard body weight of 70 kg was assumed for adults (EFSA 2012a).

Table 5: Food categories, portion sizes, percentage of lupin seeds in the food and calculated acute alkaloid intake

Food category	Portion size in g	Percentage of lupin seeds in the food	Alkaloid intake in mg/kg bw/day	Margin of safety (MOS)
Lupini beans as a snack	100	100	0.286	1
Confectionery	50	2	0.003	70
Patties	200	40	0.229	1
Muesli	100	18	0.051	4
Biscuits	50	2	0.003	70
Cakes	150	10	0.043	5
Bread	60	10	0.017	12
Spreads	30	30	0.026	8
Smoothies/Shakes	250	8	0.057	4
Ice-cream/dessert on a lupin basis	150	3	0.013	16
Dietary supplements (DS)	20	100	0.057	4

Foods containing lupin seeds in the categories “Patties” and “Lupini beans as a snack” show the highest values with regard to alkaloid intake. Acute exposure through “Lupini beans as a snack” lies at 0.286 mg/kg bw/day and through “Patties” at 0.229 mg/kg bw/day. Intake through foods of the other categories ranges from 0.003 – 0.057 mg/kg bw/day. The calculation of the MOS for the pharmacological threshold dose of 0.2 mg/kg bw/day described for sparteine produces a value of 1 for the categories “Patties” and “Lupini beans as a snack”. The MOS lies between 4 and 70 in the remaining categories.

When evaluating the results it has to be taken into account that of the 2,022 respondents, only 136 have consumed at least one of the lupin-containing foods at least once in the last 12 months. This equates to 6.7% of the respondents and shows that lupin seeds and products made from them are only consciously consumed by a small section of the population. Moreover, the data on frequency (Table 1) underscores that lupin seeds and products made from them belong to the group of foods that are rarely eaten. Those who prepare lupin-based foods by themselves use up to 70% of ready prepared products such (e.g.lupin flour) to do so. Those who buy lupin seeds as the basic product procure them up to 74% in organic shops and up to 11% in grocery stores in which mainly Turkish, Arab, Lebanese, Mediterranean or Asian foods are sold.

The chronic alkaloid intake of 0.013 mg/kg bw/day was calculated very conservatively. Where several figures were available regarding portion size or percentage of lupin seeds in the food, the larger number was always used. In addition to this, total intake over all consumers was not calculated but rather over the consumers of the foods for which the highest alkaloid intake could be recognised. For chronic intake, an alkaloid level of the lupin seeds of

60 mg/kg in products containing lupins was assumed, as the likelihood that products with levels of 200 mg/kg will always be consumed over the entire year has to be seen as low.

The acute exposure estimation produces values greater than 0.2 mg/kg bw/day in two of the 11 food groups shown. Intake via foods of the remaining categories ranges from 0.003 – 0.057 mg/kg bw/day. The MOS for the categories “Patties” and “Lupini beans as a snack” is 1 and therefore inadequate (cf. 3.1.2.3). The MOS value lies between 4 and 70 for the remaining categories. Alkaloid levels of 200 mg/kg were assumed for the acute estimation as this is the highest value that applies to sweet lupins in other countries (cf. 3.1.2.1).

The number of respondents who prepare foods from lupin seeds by themselves is too small to make an estimate of the quantity of alkaloids ingested by consumers through self-prepared foods containing lupin seeds. A comparison with data from the literature is difficult as the calculation bases are very different. The exposure estimation made by ANZFA results in an alkaloid intake of 0.002 mg/kg/day for products made on the basis of lupin seed flour. No concrete alkaloid intake quantities are mentioned for self-made foods produced from lupin seeds by consumers themselves due to the difficulty of making an estimation (ANZFA 2001). The exposure estimation made within the scope of a project commissioned by the Nordic Council of Ministers produced values of 0.32 – 0.79 mg/kg/day for adults and 0.57 – 1.4 mg/kg/day for children (Kusuhara *et al.* 2007; Pilegaard & Gry 2008). The chronic and acute alkaloid intake values calculated for Germany lie between the two values listed in the literature.

The finding made in the survey that only 21% of the consumers who know about lupin seeds are aware of the distinction between sweet and bitter lupins is also of interest for risk characterisation. All respondents who know the difference between sweet and bitter lupins and prepare sweet lupin products by themselves stated that they only use sweet lupin seeds to prepare the foods (n=4). It also had to be taken into account that low-alkaloid varieties have been cultivated and processed in Germany since the 1930s for animal and human consumption (GFL 2007). Lupin seed-based foods made from German raw materials consist of these low-alkaloid sweet lupin seeds. The designations *sweet lupin seeds, sweet lupin flour* or *sweet lupin protein* are to be found directly on the packaging of the majority of processed foods containing lupin seeds (MINTEL 2016).

Even though the market significance of lupin seeds in the human diet appears to increase, awareness of lupin seeds among the respondents is very low, so the consumption data have to be handled with care. There are no analysis values on the alkaloid content of commercial lupin seed products. The proportion of lupin seeds in the observed food groups comprises average percentages based on information provided by the manufacturers which can be higher or lower, depending on the food and brand. Furthermore, the acute exposure estimation is based on average portion sizes and on the assumption of a high alkaloid content in the lupin seeds used.

Due to the poor data base regarding lupin seeds, numerous assumptions had to be made in order to calculate exposure and this is the reason why this exposure estimation involves a comparatively high level of uncertainty.

3.1.5 Risk characterisation

Lupanine, lupinine and sparteine are listed as the main alkaloids in the seeds of *L. albus L.* (white lupin), *L. angustifolius* L. (blue lupin), *L. luteus L.* (yellow lupin) and *L. mutabilis* which are used for the production of foods. The levels of these quinolizidine alkaloid vary depend-

ing on the species and variety. Lupanine, lupinine and sparteine all have a bitter taste, with sparteine being the most bitter.

The BfR does not currently have any analysis data on the alkaloid levels of lupin seeds and products made from them which are traded as foods in Germany. Commercial foods containing lupin seeds which were examined in Italy had alkaloid levels of less than 60 mg/kg (Resta *et al.* 2008a).

It must be assumed, however, that in addition to sweet lupin seeds, bitter lupin seeds could also be in circulation the consumption of which, if they are not sufficiently debittered, could result in acute lupin alkaloid poisoning as described several times above, for which anticholinergic syndrome is typical and which can result in death through respiratory paralysis. The lowest dose which resulted in the death of a child after the consumption of lupin seeds containing over 1% sparteine and lupinine was estimated to be 11 to 22 mg lupin alkaloid/kg bw (Schmidlin-Mészáros 1973; Schmidt 1961).

An analysis of the available reports on cases of poisoning with lupin seeds showed that this was attributable repeatedly to inadequate debittering of bitter lupin seeds by consumers (Awada *et al.* 2011; Daverio *et al.* 2014; Jamali 2011; Kurzbaum *et al.* 2008; Litkey & Dailey 2007; Lowen *et al.* 1995; Smith 1987). There are no indications that the consumption of industrially produced foods on the basis of lupin seeds was associated with poisoning.

Typical symptoms of poisoning with lupin seeds are of neurological nature and/or manifest in the cardiovascular and digestive systems. Accordingly, dizziness, confusion, tachycardia, gastrointestinal complaints, nausea, mydriasis, dry mouth, loss of motor control and in high doses, bradycardia, respiratory paralysis and cardiac arrest occur.

The three main alkaloids lupanine, lupinine and sparteine produce the pharmacological and toxicological effects relevant to risk assessment. They develop inhibitory effects on nicotinic and muscarinic acetylcholine receptors, impair the function of sodium and potassium channels, have a uterine contracting effect and influence the conduction of stimuli in the heart. Quantatively, lupanine, lupinine and sparteine have similar effects, but they differ qualitatively in their efficacy potential which is most pronounced in sparteine. It has to be assumed that they mutually enhance one another in their effect.

For lupin seeds having led to cases of poisoning, above all knowledge of chemical composition is missing, in addition to an adequate characterisation of their botanical origin (variety, species). It is therefore impossible to associate the poisoning symptoms to the ingested doses of certain alkaloids or specific alkaloid pattern. There are no findings either which permit the derivation of a dose without effects in humans of the most important lupin alkaloids.

The data situation on the effects and inparticular on the dose-response relationships of the individual alkaloids and their interactions is sparse and not sufficient for the derivation of health-based guidance values (HBGV), *which, if not exceeded, ensure that no acute or chronic health impairments have to be expected.* With regard to acute toxicity, there is particularly a lack of data on the No Effect Level of the quinolizidine alkaloids regarding the induction of uterine contractions after oral exposure. What concerns longer term exposure, there is a shortage of information on the chronic, developmental toxic, reproductive toxic and genotoxic effects.

If the human data from cases of poisoning are compared with the findings of animal experiments on acute and longer-term toxicity, it becomes clear that humans quite obviously react

more sensitively to quinolizidine alkaloids than laboratory rodents do. Thus, for example, the LD_{50} for male EOPS Swiss mice with the peroral administration of sparteine is given as 220 mg/kg bw, whereas sparteine has already proven lethal for an infant in doses of roughly 30 mg/kg bw. Overall, the available studies on rats, even with administration over longer periods, would appear not adequate for risk assessment in humans.

A dose of 0.29 mg sparteine sulphate/kg bw, the equivalent of 0.20 mg sparteine/kg bw, which was described in older literature as the lowest pharmacologically effective dose and which is regarded as the threshold dose, was selected as the point of departure for the characterisation of the potential of acute hazard. In this risk assessment that has to be conducted, this dose is used as a reference value for the Margin of Safety (MOS) approach. The BfR recommends that the dose of quinolizidine alkaloids ingested with lupin seeds or foods made from them in one meal or spread over the day should lie well below the pharmacological threshold dose of 0.2 mg per kg bw described for sparteine. The MOS should take into account the uncertainty in the toxicological data base and in particular the possible higher sensitivity of children, pregnant women and poor metabolisers and should amount to more than 1.

As shown in Table 5, MOS values of 1 – 70 are calculated for acute exposure. It then becomes clear that with the food categories “Lupini beans as a snack” and “Patties”, with an assumed content of 200 mg of quinolizidine alkaloids per kg lupin seeds, quinolizidine alkaloid intake exceeds the threshold dose of 0.20 mg/kg bw assumed for sparteine. Contrary to this, an MOS ranging from 4 to 70 is calculated for the other food categories. It has to be taken into account, however, that the highest alkaloid levels measured in commercial products was 60 mg/kg lupin seeds, which would result in the trebling of the MOS values.

Chronic total lupin alkaloid intake for consumers of “Lupini beans as a snack” and “Patties” lies at 0.013 mg/kg bw. However, a toxicological reference value which could be used for the assessment of the risks of long-term exposure cannot be derived on the current level of knowledge.

From the “Consumer study on the consumption of lupin seeds” commissioned by the BfR, it can be seen that the proportion of respondents who have consciously eaten foods with lupin seeds at all is small (9.2% of respondents). The percentage of those who buy (and prepare) lupin seeds for this purpose and do not choose a processed product or lupin flour is very small (1.2% of respondents). Of the 397 respondents who knew about lupin seeds, only 21% knew the distinction between sweet and bitter lupin seeds.

It is worthy of note that there are reports in the literature of the existence of numerous “alkaloid-free” varieties of *L. albus* and *L. luteus* lkaloidfreie“ (Ternes *et al.* 2007). Seeds of the sweet lupin varieties *L. albus* nowadays have alkaloid levels under 0.05%, for example, with the best varieties reaching 50 µg/g dry weight (≙ 0.005%). The alkaloid levels of seeds of sweet lupin varieties of *L. mutabilis* can be reduced to 0.001% (Blaschek *et al.* 2016). According to the provisions that apply in other countries, it has to be assumed that comparatively low alkaloid levels which do not exceed 200 mg/kg seed, equivalent to an alkaloid content of 0.02%, can be complied with (ANZFA 2001; FSA 1996; Santé) 1998).

3.2 Conclusions and recommendations

3.2.1 Conclusions

- *Possible health risks*

Lupanine, lupinine and sparteine are described as being the main alkaloids in the seeds of *L. albus L.* (white lupin), *L. angustifolius* L. (blue lupin), *L. luteus L.* (yellow lupin) and *L. mutabilis* used in food production. Levels can vary, depending on the species and variety.

The quinolizidine alkaloids evoke typical symptoms of poisoning in humans affecting the nervous, circulatory and digestive systems. Typical symptoms of poisoning with lupin alkaloids are dizziness, confusion, tachycardia, gastrointestinal complaints, nausea, mydriasis, dry mouth, loss of motor control and in high doses, bradycardia, cardiac arrest and respiratory paralysis.

The dose of quinolizidine alkaloids ingested with lupin seeds or foods made from them in one meal or spread over the day should lie well below the pharmacological threshold dose of 0.2 mg per kg bw described for sparteine. The MOS should take into account the uncertainty in the toxicological data base and in particular the possible higher sensitivity of children, pregnant women and poor metabolisers. Overall, a margin of safety (MOS) of 1 is regarded as insufficient.

An analysis of the available reports on cases of poisoning through lupin seeds showed no indications of an association with the intake of industrially produced foods made on the basis of lupin seeds. Relationships were seen repeatedly, however, between cases of poisoning by the inadequate processing of the lupin seeds (insufficient debittering) and subsequent consumption of foods prepared by consumers themselves from lupin seeds.

- *Analytics of alkaloid levels in lupin seeds*

Only a few analytical methods have been described by means of which the various alkaloids can be quantified. None of the methods have been validated in a method validation study.

For this reason, the BfR does not currently have any analytical data on the alkaloid levels of lupin seeds and products made from them which are traded as foods in Germany. It must be assumed, however, that bitter lupin seeds could be in circulation the consumption of which could lead to acute lupin alkaloid poisoning as described above in cases of inadequate debittering.

- *Data on consumer exposure*

Acute exposure estimates assume an alkaloid content of 200 mg/kg seeds. This produces the highest alkaloid intake values for foods containing lupin seeds in the categories “Lupini beans as a snack” (0.286 mg/kg bw/day) and “Patties” (0.229 mg/kg bw/day). Intake via foods in the other categories ranges from 0.003 – 0.057 mg/kg bw/day. During risk assessment, these alkaloid intakes are compared with the pharmacological threshold dose of 0.2 mg/kg bw described for sparteine as a reference value. The MOS to the threshold dose should take into account the uncertainty in the toxicological data base and in particular the possible higher sensitivity of children, pregnant women and poor metabolisers and should amount to more than 1. The MOS of 1 that

results for the first two categories is regarded as insufficient. The MOS for the remaining categories lies between 4 and 70.

Chronic exposure estimates assume an alkaloid content of 60 mg/kg seeds. It can be seen here too that foods in the categories “Lupini beans as a snack” and “Patties” result in the highest alkaloid intake. Chronic total alkaloid intake is identical for the consumers of foods in both of these categories and amounts to 0.013 mg/kg bw/day. A toxicological reference value which could be used for the assessment of the risks of longer-term exposure cannot be derived with the current level of knowledge.

It has to be considered that in accordance with the “Consumer survey on the consumption of lupin seeds” commissioned by the BfR, the conscious consumption of lupin seeds in Germany is comparatively low. Therefore no sufficient knowledge can be assumed among consumers for the differences between sweet and bitter lupin seeds and how to prepare them, e.g. of the special processes required to debitter (remove the alkaloids from) bitter lupin seeds,.

Where home debittering processes are concerned, varying methods have been described most of which involve boiling the seeds and soaking them for several days with multiple changes of the water. There are no systematic and validated examinations of the quality of these methods, however, the success of which also depends on the variable initial alkaloid levels in the seeds and cases of poisoning were repeatedly attributable to the inadequate debittering of bitter lupin seeds by consumers. Thus, BfR cannot make a general recommendation here either.Research results of the industrial debittering of lupin seeds are described in the literature. These methods are not subject of this opinion.

3.2.2 Recommendations

From the data base which has been described in detail, the following recommendations have to be made in the view of the BfR:

- *Recommendations related to the toxicology, analytics and exposure*

Comprehensive chemical and toxicological characterisation of the lupin seeds of different botanical provenance sold as foods is regarded as necessary.

The present knowledge of doses without effects on humans is insufficient, concerning the anticholinergic effects of quinolizidine alkaloids. A need for research is seen in particular with regard to the existing dose-response relationships of the potentially uterus-contracting effect of lupanine, lupinine and sparteine after oral administration and with regard to the potential developmental toxic effect of certain lupin alkaloids, such as ammodendrine and multiflorin and their derivatives, which occur mainly in wild lupins and the occurrence of which in lupin seeds intended for human consumption is undesired.

For the characterisation of lupin seeds of various botanical origin offered as food, the preparation of the alkaloid profile should include the varieties used in the food area, as well as lupin alkaloids such as anagyrine, ammodendrine and multiflorin and their derivatives for which a potential of developmental toxicity is suspected due to cases of poisoning in animals (3.1.1 (a)).

For this the preparation of adequate analytical methods for alkaloid determination (in-house validation) and the release of suitable certified reference materials are regarded as necessary. The methods should be accredited in line with DIN EN ISO 17025.

Data on the alkaloid levels in industrially produced lupin seed products should be collected for exposure estimation. Where exposure estimation is concerned, the BfR recommends that a European approach that is uniform throughout the EU be proposed with which the different consumption habits in the various EU member states can be taken into account.

- *Recommendations in connection with marketing*

When marketing whole, uncrushed lupin seeds intended for direct processing and/or direct consumption by the consumer (snacks) , it is recommended that only whole, uncrushed lupin seeds are brought into the market which can be consumed without the need for any debittering processes by the consumer. These can be sweet lupin seeds with a natural low alkaloid content or bitter lupin seeds which were sufficiently debittered prior to marketing.

In the light of the possible toxic effects caused by lupin alkaloids, it is important to emphasise, that when bringing lupin seed flour intended for human consumption into the market, it should be ensured that the flour was made from lupin seeds which were low in alkaloids and/or sufficiently debittered.

With lupin seeds and products derived from them which are used by manufacturers for industrial further processing, it should be ensured that they are low in alkaloids and/or that they have been sufficiently debittered.

- *Recommendations for consumers*

If lupin seeds or products made from them have a bitter taste, this can be an indicator for the presence of undesired, health-damaging lupin alkaloids. The bitter-tasting water used to soak the lupin seeds should not be consumed under any circumstances or used for the preparation of foods.

As a precaution, it is recommended that consumers avoid the consumption of bitter lupin seeds which were not debittered by the manufacturer, as there is no certainty that the recommended debittering procedures result in a sufficient reduction in the levels of health-damaging alkaloids.

More information at the BfR website on the subject of lupin seeds:

BfR opinion “Allergies Through Lupin Protein in Foods” (German only):
http://www.bfr.bund.de/cm/343/allergie-durch-lupineneiweiss-in-lebensmitteln.pdf

BfR brochure “Risk Plant – Estimation and Notes” (German only):
http://www.bfr.bund.de/cm/350/risiko-pflanze-einschaetzung-und-hinweise.pdf

BfR brochure “Risk – Cases of Poisoning in Children” (German only):
http://www.bfr.bund.de/cm/350/risiko-vergiftungsunfaelle-bei-kindern.pdf

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