Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products

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Abstract

The European Commission asked EFSA for a scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids (QAs) in feed and food. This risk assessment is limited to QAs occurring in Lupinus species/varieties relevant for animal and human consumption in Europe (i.e. Lupinus albus L., Lupinus angustifolius L., Lupinus luteus L. and Lupinus mutabilis Sweet). Information on the toxicity of QAs in animals and humans is limited. Following acute exposure to sparteine (reference compound), anticholinergic effects and changes in cardiac electric conductivity are considered to be critical for human hazard characterisation. The CONTAM Panel used a margin of exposure (MOE) approach identifying a lowest single oral effective dose of 0.16 mg sparteine/kg body weight as reference point to characterise the risk following acute exposure. No reference point could be identified to characterise the risk of chronic exposure. Because of similar modes of action for QAs, the CONTAM Panel used a group approach assuming dose additivity. For food, the highest mean concentration of Total QAs (TotQAs) (i.e. the 6 most abundant QAs) was found in lupin seed samples classified as ’Lupins (dry) and similar’-1. Due to the limited data on occurrence and consumption, dietary exposure was calculated for some specific scenarios and no full human health risk characterisation was possible. The calculated margin of exposures (MOEs) may indicate a risk for some consumers. For example, when lupin seeds are consumed without a debittering step, or as debittered lupin seeds high in QA content and when ’lupin-based meat imitates’ are consumed. For horses, companion and farm animals, other than salmonids, the available database on adverse effects was too limited to identify no-observed-adverse-effect levels and/or lowest-observed-adverse-effect levels and no risk characterisation was possible. For salmonids, the CONTAM Panel considers the risk for adverse effects to be low.

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Keywords: Lupin, quinolizidine alkaloid, sparteine, lupanine, margin of exposure (MOE), food, feed

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Summary

The European Commission asked the European Food Safety Authority (EFSA) for a scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids (QAs) in feed and food, in particular in lupins and lupin derived products. The CONTAM Panel provided an opinion that comprised:

- information on QAs occurring in *Lupinus* species and varieties that are relevant for animal and human consumption in Europe. These are *Lupinus albus* L. (white), *Lupinus angustifolius* L. (blue or narrow-leaved), *Lupinus luteus* L. (yellow) and *Lupinus mutabilis* Sweet (Andean or pearl). In particular the present assessment is confined to the evaluation of the most relevant QAs present in the seeds of these lupin species (i.e. angustifoline, \( \gamma \)-isolupanine, lupanine, 13\( \alpha \)-OH-lupanine, lupinine, multiflorine, sparteine, tetrahydrorhombifoline, 13\( \alpha \)-tigloyloxylupanine).
- evaluation of the toxicity of these QAs considering all the relevant toxicological endpoints and assessment of the human and animal health risks due to the estimated dietary exposure to these QAs of the EU population and farm animals, horses and companion animals.

Information on the toxicokinetics and metabolism of QAs in humans and experimental animals is limited to sparteine and lupanine. Both compounds are rapidly absorbed, widely distributed and rapidly eliminated in urine mainly unchanged or as oxidised metabolites. Genetic polymorphisms in CYP2D6 (occurring in 5–10% of Caucasians, also called poor metabolisers) may affect sparteine oxidation in humans leading to a slower elimination. Similarly, scarce data are available on ‘absorption, distribution, metabolism, and excretion’ (ADME) in farm animals, horses and companion animals. In ruminants, there is extensive absorption and slow elimination of lupanine and 5,6-dehydrolupanine. In pigs, lupanine is extensively absorbed and excreted in urine mainly unmodified, together with the metabolites isolupanine and 13-OH-lupanine. There is no evidence of the formation of conjugated metabolites of sparteine, lupanine or their metabolites in any species. No information is available to assess transfer rates of QAs in food of animal origin; however, based on indirect evidence, possible transfer to milk should be considered.

Acute toxicity studies in mice and guinea pigs indicate that sparteine is approximately two- to three-fold more toxic than lupanine. In rats, lupanine and 13\( \alpha \)-OH-lupanine have a similar toxicity. Mice appear to be more sensitive than rats to acute toxicity of lupanine. Notwithstanding the differences in toxicities of sparteine, lupanine and 13\( \alpha \)-OH-lupanine, the acute symptoms following oral administration of these QAs are similar in all species, with death resulting from respiratory failure and toxic effects affecting the central nervous system (CNS).

None of the repeated dose studies on QAs were carried out using pure compounds, but they were all based on diets containing seed extracts or flour from various lupin species and were all performed in the rat. These studies could not be used for the evaluation of QA toxicity.

Angustifoline, 13\( \alpha \)-OH-lupanine, lupanine, lupinine, sparteine, 13\( \alpha \)-tigloyloxylupanine, cytisine, 3\( \beta \)-OH-lupanine, 17-oxosparteine were unable to bind or intercalate into DNA. Lupanine and extracts of *Lupinus termis* (the main QA being lupanine) did not induce gene mutations in standard bacterial assays and in mammalian cells *in vitro* or micronuclei *in vivo*. The data on sparteine are too limited to conclude regarding genotoxicity.

In humans, a large part of the information on the toxicity of QAs is confined to sparteine, mainly because of its therapeutic use in the past as an antiarrhythmic and oxytocic drug. In the treatment of cardiac arrhythmia, the recommended daily dosage for (-)-sparteine sulfate was in the range of 800–1,000 and 400–500 mg/day for short-term and for long-term therapy, respectively. Adverse effects of anticholinergic nature were reported at these therapeutic doses. The lowest dose mentioned with antiarrhythmic effects was 20 mg (equivalent to 0.16 mg sparteine/kg body weight (bw) for a person with a body weight of 70 kg). Individual variations in sensitivity to sparteine sulfate are explained by the genetic polymorphisms in CYP2D6, with poor metabolisers being considered as a group at higher risk to develop side effects. When sparteine sulfate was given as a single oral dose of 100–150 mg to pregnant women because of its oxytocic properties, vomiting was the only adverse effect reported.

The symptoms of intoxication with sparteine are dizziness, drowsiness, headache, sweating, mydriasis and myasthenia. Sparteine blocks the transmission of signals from the nerves to the muscles like curare, which may lead to respiratory arrest and death. From a poisoning case, it is known that a dose of 30 mg of sparteine/kg bw was fatal to a young child. No adverse effects were observed after administration to volunteers (including poor metabolisers) of single oral doses of 10 mg of either...
lupanine or 13\textsubscript{-}OH-lupanine (each equivalent to 0.14 mg/kg bw for a person with a body weight of 70 kg).

Human cases of poisoning with lupin seeds are infrequent and rarely lead to a fatal outcome. However, case reports of poisoning in children suggest that these are more susceptible to intoxication than adults, and doses of lupin alkaloids from 10 mg/kg bw onwards may be lethal. Intoxication has been associated with inadequate debittering of lupin seeds by consumers, while no case of poisoning has been associated with consumption of industrially produced lupin seed-based food.

A study designed to determine the lethality of selected QAs in laying hens confirmed the higher acute toxicity (two- to three-fold) of sparteine in comparison to lupanine reported in mammals. The large majority of relevant repeated-dose studies in livestock species were not performed to investigate the toxicity of QAs, but aimed at studying the lupin impact on zootechnical performances (feed intake, weight gain, milk and egg production). Lack of specific toxicity studies together with considerable differences in total and specific QA content among lupin seeds from different species/cultivars did not allow identification of a no-observed-adverse-effect level (NOAEL)/lowest-observed-adverse-effect level (LOAEL) for most food producing species. These were replaced by suggested tolerated concentrations/doses specifically referring to seeds from a single lupin species/cultivar. The only studies performed with pure QAs (i.e. lupinine and sparteine) were conducted in rainbow trout and allowed identifying similar NOAEL values for lupinine and sparteine (1 mg/kg bw per day) and a LOAEL of 2.5 mg/kg bw per day for lupinine and 3.5 mg/kg bw per day for sparteine. No relevant studies have been identified for horses and companion animals.

Several modes of action have been identified for QAs, the most important acute toxic effect being the inhibitory action on acetylcholine receptors (AChRs) in the CNS, in the peripheral autonomic system and on the motor endplates. This produces an anticholinergic syndrome with paralysis of respiratory muscles causing respiratory failure and death. The different QAs appear to bind with different affinity to AChRs and also their preference for nicotinic AChRs (nAChRs) or muscarinic AChRs (mAChRs) seems to differ. QAs may also cause, with different potencies, inhibition of voltage-dependent ion channels, with these effects possibly underlying their antiarrhythmic effect. The uterotonic effects of sparteine appear to be mediated via an increased production of prostaglandin F. Although most data are on sparteine, affinities to AChRs of other QAs relevant in this opinion appear to be in the same range or lower than those of sparteine. Scarce data on other modes of action of a few QAs indicate that these QAs are similarly or less active than sparteine. No studies investigating possible additive effect of QAs were identified.

The anticholinergic effects and the effects on the electrical conductivity of the heart following acute exposure to QAs are the critical effects for the human hazard characterisation. The mode of action of sparteine and its pharmacological profile as an anticholinergic substance are considered similar to those of other main alkaloids in lupin seeds (lupanine, 13\textsubscript{-}OH-lupanine and lupinine). Therefore, the CONTAM Panel considers it appropriate to use a group approach and apply the principle of dose additivity with equal potencies of the QAs for their combined effect. In the absence of dose–response information for other QAs, the CONTAM Panel decided to base the hazard characterisation on sparteine, which has a higher potency than other QAs. The available data were considered insufficient to propose health-based guidance values (HBGVs) and instead an MOE approach using the lowest single oral active dose of 0.16 mg sparteine/kg bw as reference point was selected to characterise the risk following acute exposure. No reference point could be identified to characterise the risk following chronic exposure.

The occurrence of QAs in lupin-based food samples was estimated using a data set of 1,035 analytical results from 99 food samples provided by 2 data providers. Information on the lupin species was reported only in some cases, with the samples being equally distributed between 

L. albus

and

L. angustifolius

Lupanine was the most abundant QA in seeds from these lupin species followed by 13\textsubscript{-}OH-lupanine and angustifoline. Total QAs (TotQAs) as the sum of the 6 most abundant QAs present in the data set (lupanine, 13\textsubscript{-}OH-lupanine, angustifoline, multiformine, 13\textsubscript{-}tigloyloxylupanine and 13\textsubscript{-}isolupanine) were also calculated. The highest mean concentration of TotQAs was found in lupin seed samples classified as ‘Lupins (dry) and similar’ (429 mg/kg). These are sold as dry seeds and can be consumed upon rehydration, debittering, boiling or as flour for home-made dishes (pancakes/crepes or pasta). High mean concentrations of TotQAs were also found in ‘lupin-based coffee imitates ingredients’ (331 mg/kg), while lower values (> 10-fold) were found in ‘canned or jarred legumes’, ‘meat imitates’, or ‘pasta plain uncooked’.

Lupin seeds with a high concentration of QAs need to be debittered before consumption. Between 89 and 97% of the QAs present in seeds are removed by water treatment and boiling. Because of the...
limited amount of data on occurrence and consumption, the CONTAM Panel decided to calculate acute dietary exposures for some specific food groups under specific scenarios. In a first scenario, exposure due to lupin seed consumption was estimated but it was assumed that no reduction of the QA content occurred because of the lack of debittering of dry lupin seeds by the consumer. The P95 acute dietary exposure was 761 and 1,700 μg/kg bw per day for lupin seeds with average and high TotQA content. In a second scenario, the concentration of TotQA was assumed to decrease by 89% following soaking and boiling in water of the lupin seeds and P95 exposures were 84 and 187 μg/kg bw per day for an average and high QA content, respectively. The third scenario was based on ready-to-eat lupin seeds and resulted in P95 exposures of 44–46 μg/kg bw per day (lower bound (LB)–upper bound (UB)).

Higher P95 dietary exposures of 194–228 μg/kg bw per day (LB–UB) were reached in the fourth scenario reflecting consumption of lupin-based meat imitates. All these scenarios reflect the exposure of adults and scenarios 1, 2 and 3 could not be calculated for other age groups due to limitations in the consumption data. In addition, exposure from certain lupin-based foods such as pasta, bread and coffee imitates could not be assessed. Consequently, no full risk characterisation was possible.

There is uncertainty linked to the reference point of 160 μg sparteine/kg bw. This reference point for QAs is based on the effects of sparteine which is the most potent QA with respect to toxicity. However, sparteine occurs only in trace amounts according to the data submitted to EFSA and, according to clinical experience, doses 7.5–10 times higher than the reference point ensure a reliable antiarrhythmic effect. Therefore, the CONTAM Panel concluded that MOEs > 1 do not indicate a health concern. In the event that lupin seeds were not debittered and for a scenario reflecting dietary exposure from lupin-based meat imitates the CONTAM Panel calculated MOE values in the range of 0.1–0.8. These MOEs may indicate a concern. In a scenario applying a reduction factor for debittering, the calculated MOEs are of lower concern (0.9; high QA concentration) or no concern (1.9; average QA concentration). MOEs > 1 were calculated for ready-to-eat lupin seeds that had an average QA content.

Dietary exposure of farm animals, horses and companion animals was estimated using a data set containing both feed and food samples (n = 54) provided by two data providers. Lupanine and 13α-OH-lupanine accounted for about 36% of the TotQA in these samples. Exposures to QAs by farm animals, horses and companion animals were estimated using maximum recommended inclusion rates of lupin seeds in livestock diets and represent worst-case scenarios. In addition, insufficient data on levels of QAs in lupin seeds were available to allow P95 estimates of exposure to be made. In the category ‘Ruminants and horses’, the highest exposure was for lactating goats (3.3 mg/kg bw per day). For pigs and poultry, the highest exposure was for fattening chickens and laying hens (3.5 mg/kg bw per day). For salmonids, the exposure was 1.6 mg/kg bw per day and for rabbits 4.4 mg/kg bw per day. For cats and dogs the exposure was 0.6 mg/kg bw per day.

For the animal health risk characterisation, the CONTAM Panel applied a group approach for salmonids and used a NOAEL of 1 mg/kg bw per day for the relevant QAs in this opinion. The mean dietary exposure of salmonids is above the NOAEL but below the LOAEL of 2.5 mg/kg bw per day. The CONTAM Panel considers the risk for adverse effects in salmonids to be low. For the other species, the available database on adverse effects was too limited to identify overall NOAEL/LOAELs. In an alternative approach, the CONTAM Panel identified doses of QAs present in lupin seeds that can be tolerated with respect to zootechnical performance. However, such a dose is only applicable to lupin seeds with similar QA composition and does not allow a full characterisation of the risk. For cattle and rabbits, the mean dietary exposure is well below the tolerated dose. For pigs and chickens, the mean dietary exposure in the same range as the dose that can be tolerated.

This risk assessment relates to lupin seeds and lupin-derived products with a QA profile comparable with that of the submitted data and should not be extrapolated to lupin seeds with a different QA profile (e.g. lupin seeds from species and/or varieties high in sparteine).

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of QAs in lupin seeds and lupin-derived products is substantial due to the limited data on toxicity, occurrence and consumption. The CONTAM Panel recommends the generation of more data related to the toxicokinetics, toxicity and occurrence of QAs as well as to the consumption of lupin seeds and lupin-based foods in order to refine the risk assessment.
Table of contents

Abstract ................................................................................................................................................. 1
Summary ............................................................................................................................................... 3
1. Introduction ..................................................................................................................................... 8
1.1. Background and Terms of Reference as provided by the requestor ............................................. 8
1.2. Interpretation of the Terms of Reference .................................................................................... 8
1.3. Supporting information for the assessment ................................................................................. 8
1.3.1. Chemistry .................................................................................................................................. 8
1.3.2. Analytical methods .................................................................................................................... 9
1.3.3. Botanical origin, varieties and cultivation ............................................................................... 10
1.3.4. Previous risk assessments ....................................................................................................... 15
1.3.5. Legislation ................................................................................................................................. 16
1.2. Interpretation of the Terms of Reference ...................................................................................... 8
1.3. Supporting information for the assessment ................................................................................... 8
1.3.1. Chemistry .................................................................................................................................. 8
1.3.2. Analytical methods .................................................................................................................... 9
1.3.3. Botanical origin, varieties and cultivation ............................................................................... 10
1.3.4. Previous risk assessments ....................................................................................................... 15
1.3.5. Legislation ................................................................................................................................. 16

2. Data and methodologies ............................................................................................................. 17
2.1. Methodology for data collection and study appraisal ................................................................. 17
2.2. Food and feed occurrence data submitted to EFSA ................................................................... 18
2.2.1. Data collection ........................................................................................................................ 18
2.2.2. Data validation and analysis ................................................................................................... 18
2.3. Food and feed consumption data .............................................................................................. 18
2.3.1. Food consumption .................................................................................................................. 18
2.3.2. Feed consumption .................................................................................................................. 19
2.3.3. Food classification ................................................................................................................... 19
2.3.4. Feed classification ................................................................................................................... 19
2.4. Methodology for exposure assessment ....................................................................................... 19
2.4.1. Human exposure assessment .................................................................................................. 19
2.4.2. Animal exposure assessment .................................................................................................. 20
2.5. Methodology for risk characterisation ....................................................................................... 20
3. Assessment ................................................................................................................................... 20
3.1. Hazard identification and characterisation .................................................................................. 20
3.1.1. Toxikokinetics and metabolism .............................................................................................. 20
3.1.1.1. Laboratory animals and humans .......................................................................................... 20
3.1.1.2. Farm animals, horses and companion animals ................................................................ 21
3.1.1.3. Transfer ............................................................................................................................. 24
3.1.1.4. Main biotransformation pathways of quinolizidine alkaloids .............................................. 24
3.1.2. Summary remarks on toxicokinetics ....................................................................................... 24
3.1.2.1. Acute toxicity ...................................................................................................................... 25
3.1.2.2. Repeated dose toxicity studies ........................................................................................... 26
3.1.2.3. Neurotoxicity ..................................................................................................................... 28
3.1.2.4. Genotoxicity ....................................................................................................................... 29
3.1.2.5. Developmental and teratogenicity studies ....................................................................... 30
3.1.2.6. Carcinogenicity .................................................................................................................. 30
3.1.3. Observations in humans .......................................................................................................... 30
3.1.3.1. Pharmacodynamics .......................................................................................................... 31
3.1.3.2. Human studies .................................................................................................................... 32
3.1.3.3. Case reports on intoxication with lupin seeds ................................................................... 32
3.1.4. Adverse effects in farm animals, horses and companion animals ......................................... 34
3.1.4.1. Ruminants .......................................................................................................................... 34
3.1.4.2. Pigs ..................................................................................................................................... 35
3.1.4.3. Poultry ............................................................................................................................... 37
3.1.4.4. Rabbits ............................................................................................................................... 39
3.1.4.5. Fish ..................................................................................................................................... 39
3.1.4.6. Horses ................................................................................................................................ 40
3.1.4.7. Companion animals .......................................................................................................... 40
3.1.4.8. Summary remarks ............................................................................................................. 40
3.1.5. Mode of action ........................................................................................................................ 41
3.1.6. Consideration of critical effects and dose-response analysis .................................................... 42
3.1.6.1. Acute toxicity ..................................................................................................................... 44
3.1.6.2. Repeated-dose toxicity ....................................................................................................... 45
3.1.7. Possibilities for derivation of a human health-based guidance value (HBGV) ......................... 46
3.2. Occurrence data .......................................................................................................................... 46
Quinolizidine alkaloids in feed and food

3.2.1. Current occurrence data submitted to EFSA ......................................................... 46
3.2.1.1. Occurrence data on food .................................................................................. 47
3.2.1.2. Occurrence data on feed .................................................................................. 49
3.2.1.3. Relative variations of main QAs in lupin seeds .................................................. 50
3.2.1.4. Occurrence data received during the public consultation .................................. 52
3.2.2. Previously reported occurrence data in the open literature ................................. 52
3.2.3. Factors influencing the concentration of quinolizidine alkaloids/Food and feed processing ......................................................... 53
3.2.3.1. Debittering of lupin seeds .............................................................................. 53
3.2.3.2. Changes during production of processed food .................................................. 54
3.3. Exposure assessment ............................................................................................... 54
3.3.1. Current dietary exposure assessment for humans ................................................. 54
3.3.1.1. Occurrence data and scenarios definition ......................................................... 54
3.3.1.2. Consumption ................................................................................................. 55
3.3.1.3. Results .......................................................................................................... 56
3.3.1.4. Consumption of lupin-based feed in livestock .................................................. 56
3.3.1.5. Consumption of lupin-based feed in companion animals .................................. 57
3.3.1.6. Omission of lupin-based feed in livestock ....................................................... 57
3.3.1.7. Omission of lupin-based feed in farm animals, horses and companion animals 58
3.3.1.8. Summary of results ....................................................................................... 58
3.3.1.9. Other uncertainties ....................................................................................... 59
3.3.2. Exposure scenario/exposure model ...................................................................... 59
3.3.2.1. Assessment objectives ..................................................................................... 60
3.3.2.2. Model input (parameters) ............................................................................... 60
3.3.2.3. Other uncertainties ....................................................................................... 61
3.3.2.4. Summary of uncertainties ............................................................................. 61
4. Conclusions ................................................................................................................. 62
4.1. Hazard identification and characterisation ............................................................... 62
4.1.1. Toxicokinetics ................................................................................................... 62
4.1.2. Toxicity in laboratory animals ........................................................................... 62
4.1.3. Observations in humans .................................................................................... 62
4.1.4. Farm animals, horses and companion animals ..................................................... 63
4.1.5. Mode of action ................................................................................................. 63
4.1.6. Possibilities for derivation of a HBGV ............................................................... 63
4.2. Occurrence and exposure ....................................................................................... 64
4.2.1. Food .................................................................................................................. 64
4.2.2. Feed .................................................................................................................. 64
4.3. Risk characterisation .............................................................................................. 64
4.3.1. Human health risk characterisation ..................................................................... 64
4.3.2. Animal health risk characterisation ..................................................................... 65
4.4. Recommendations .................................................................................................. 65

Documentation provided to EFSA .................................................................................. 65

References ....................................................................................................................... 65

Abbreviations .................................................................................................................. 74

Appendix A – Major quinolizidine alkaloids present in Lupinus and related genera from the Genistae tribe ......................................................... 77
Appendix B – Other relevant alkaloids present in Lupinus and related genera from the Genistae tribe ......................................................... 81
Appendix C – Biosynthetic pathway of ester-type quinolizidine alkaloids in Lupinus species (Bunsupa et al., 2013) .............................................. 82
Appendix D – Occurrence of QAs in lupin seeds ............................................................. 83
Appendix E – Identification and selection of relevant scientific literature and reports ................................................................. 94
Appendix F – EFSA guidance documents on risk assessment ........................................ 95
Appendix G – Feed intakes and diet composition (livestock) ........................................ 96
Appendix H – Hazard identification and characterisation ............................................. 103
Annex A – Dietary surveys and occurrence data in food submitted to EFSA ..................... 113
Annex B – Occurrence data in feed submitted to EFSA ................................................ 113
Annex C – Results of acute dietary exposure assessment to quinolizidine alkaloids .......... 113
1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Background

Quinolizidine alkaloids (QAs) mainly occur in lupin species and other plants of the Genistae tribe. They are secondary metabolites for defense against pathogens and other predators. QAs are biosynthesized from lysine in green tissues of the plant and stored in all organs of the plant, including seeds (Boschin and Resta, 2013).

The level of QAs depends on genotype, presence of pathogens, environmental effects and soil characteristics. More than 170 QAs have been identified in different *Lupinus* species and the alkaloid pattern is highly variable among different species (Boschin and Resta, 2013).

Lupins and lupin-derived products are listed in the Catalogue of feed materials.1

Lupin-based products, such as lupin proteins, are gaining attention to replace animal proteins and other plants ingredients in several foods such as bakery products, imitation dairy and meat products, and beverages.

Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the risks for animal and human health related to the presence of QAs in feed and food, in particular in lupins and lupin derived products.

1.2. Interpretation of the Terms of Reference

More than 500 lupin species, comprising wild and domesticated species, have been described worldwide (Wink et al., 1995). Lupin species contain so called lupin alkaloids as important secondary metabolites. ‘Lupin alkaloids’ is a general term that comprises the QAs, but also other alkaloids such as piperidines (e.g. ammodendrine) and indoles (e.g. gramine). Lupins also contain other antinutritional factors such as phytic acid, saponins and tannins. Following the terms of reference, the present assessment is confined to the evaluation of QAs. Furthermore, only QAs occurring in lupin species and varieties that are relevant for animal and human consumption in Europe (i.e. *Lupinus albus* (white lupin), *Lupinus angustifolius* (blue or narrow-leaved lupin), *Lupinus luteus* (yellow lupin) and *Lupinus mutabilis* (Andean or pearl lupin)) are considered in this Scientific Opinion. The allergenic potential of lupin proteins has been addressed in previous EFSA opinions (EFSA, 2006; EFSA NDA Panel, 2014) and is also not dealt with in the present evaluation. Lupins are the main host of the fungus *Diaporthe toxica*, which produces phomopsins. Phomopsins are a family of mycotoxins that has been evaluated by EFSA in a previous opinion (EFSA CONTAM Panel, 2012) and are also considered not part of the present assessment.

1.3. Supporting information for the assessment

1.3.1. Chemistry

QAs are a broad group of secondary metabolites present in all species of the genus *Lupinus* and related genera from the Genistae tribe. Biosynthetically, QAs are derived from lysine that is converted to cadaverine, which is the central intermediate from which all QAs are formed. QAs have quinolizidine as a core structure that consists of two fused 6-membered rings with a nitrogen atom at the bridgehead (see Figure 1). QAs found in lupin can have a bicyclic, tricyclic or tetracyclic structure. The 11 QAs that are considered most relevant with respect to occurrence in lupin species for human and animal consumption in Europe are shown in Figure 1. A more comprehensive overview of QA chemical structures and some physical properties of QAs present in the genus *Lupinus* are listed in Appendix A. Some other alkaloids found in lupin species are also listed in Appendix B.

With respect to the stereochemistry of sparteine, lupanine and lupinine, (-)-sparteine was detected in *L. luteus* and other lupin species (Merck Index, 2006; Blaschek et al., 2006), (+)-lupanine was found in *L. angustifolius* and racemic lupanine in *L. albus* (Merck Index, 2006) and (+)-lupinine was identified in *L. luteus* and other lupin species (Merck Index, 2006). It should be noted that in cases

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1 Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1.
where the stereochemical configuration of sparteine, lupanine and lupinine is not indicated in the text, it was not defined in the underlying reference.

1.3.2. Analytical methods

Extraction and purification

QAs can be extracted from ground lupin seeds with acidic solutions (0.1–1 N HCl or trichloroacetic acid) (Wink et al., 1995; Boschin et al., 2008; Kamel et al., 2016), optionally preceded by a hexane defatting step (Boschin et al., 2008). Liquid–liquid extraction of alkaline or alkalinised extracts with chloroform or dichloromethane using Extrelut columns yields an enriched extract containing the QAs (Wink et al., 1995; Boschin et al., 2008). Although less frequently, dilute sodium hydroxide solutions have also been used to extract QAs from the seeds (Hatzold et al., 1983). Direct extraction of ground seeds by maceration in the presence of organic solvents such as chloroform and diethyl ether has also been described (Pothier et al., 1998).

Titration and TLC methods

The total alkaloid content in seeds can be estimated by titration of the QAs in their salt free form, with p-toluenesulfonic acid in combination with a pH colour indicator, e.g. tetrabromophenolphthalein ethyl ester (Ruiz, 1977) or bromocresol green (Ruiz et al., 1977). The limit of quantification (LOQ) of these methods is around 100 mg/kg seeds (Ruiz, 1978). Chloroform extracts obtained by the extraction procedure described above, upon titration gave a reasonable indication of the total alkaloid content (Ruiz, 1978). Nevertheless, a drawback of the method is that it will determine QAs together with other co-extracted alkaloids present in lupin seeds.

Thin-layer chromatography (TLC) can be used to obtain qualitative information on the QA composition of lupin seeds. Separation on silica gel plates and visualisation can be accomplished with Dragendorff’s reagent and ultraviolet (UV) light (Muzquiz et al., 1994). A quantitative 2D-TLC method has been described by Karlsson and Peter (1978).

GC-based methods

Analytical methods based on capillary high resolution gas chromatography (GC) have been in widespread use since the early 1980s. Notably the research group of Wink has published a large number of papers on the analysis of lupin seeds and other plant parts using GC methods, culminating in their overview paper of 1995, in which the spectral data and QA compositions for 56 different lupin

Figure 1: Quinolizidine core structure and the most relevant quinolizidine alkaloids for human and animal consumption in Europe found in Lupinus species
species are presented (Wink et al., 1995). In essence, the methods in use today are still very similar to the methods developed in the 1980s. Typically GC systems are equipped with either an aspecific flame ionisation detector (FID), a nitrogen specific detector (nitrogen-phosphorus detector, NPD) or they are coupled to a mass spectrometer (MS), typically generating electron impact (EI) spectra (Boschin and Resta, 2013). By using specific non-polar stationary phases (typically HP-1 (DB-1) or HP-5 (DB-5)) in combination with Kovats retention indexes, databases containing spectral information on a large number of QAs have been made available (Wink et al., 1995; National Institute of Standards and Technology (NIST) database). Detection limits (LOD) are generally not reported, as they are primarily considered sufficient for the analysis of lupin seeds and other plant products. However, the LOQ of the method becomes relevant for analysis of food products containing lupin seeds as an ingredient or for processed foods. Resta et al. (2008) and Boschin et al. (2008) reported for lupanine an LOD of 2 mg/kg (LOD = 1 mg/kg) in flour. Reinhard et al. (2006) reported for lupanine, angustifoline and 13α-OH-lupanine, LODs of 1, 2 and 6 mg/kg, respectively.

Derivation of QAs is not necessary for analysis of lupin seeds, but it is sometimes used to obtain lower LODs, particularly in complex matrices, such as food (Reinhard et al., 2006). After derivatisation, the LOD for angustifoline improved to 0.3 mg/kg and for 13α-OH-lupanine to 0.5 mg/kg.

Due to the lack of available reference standards, quantification is often performed by comparison of relative peak areas to that of an available standard, e.g. sparteine or lupanine (Boschin and Resta, 2013). This may introduce considerable uncertainty in the reported concentrations (Resta et al., 2008).

**LC-based methods**

Liquid chromatography (LC)-based methods are not often used for the determination of QAs. Wysocka and Chrzanowska (2004) used an high-performance liquid chromatography (HPLC) method with a chiral analytical column and UV detection at 220 nm to study the enantiomeric purity of lupanine in L. albus and L. angustifolius cultivars. The (+)- and (-)-enantiomers of lupanine could be separated on a Chiralcel OD column (Wysocka and Chrzanowska, 2004; Przybyl and Kubicki, 2011). Green et al. (2015a) used a liquid chromatography coupled mass spectrometry (LC-MS) method to quantify the major QAs of Lupinus leucophyllus (anagyrine, lupanine, 5,6-dehydrolupanine and an unidentified QA with mass 248) in serum of Holstein cows that had been orally dosed with dried L. leucophyllus plant material. The estimated LOQs were around 10 ng/mL in serum. Sparteine, lupanine and lupinine are sometimes included in multi analyte methods covering a wider range of plant toxins (Mol et al., 2011; Carlier et al., 2015). A conference abstract reports on the development of a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the major QAs in lupin flour (Mulder and de Nijs, 2015). QAs were extracted from the flour with an acidic aqueous solvent. The extracts were analysed without further clean-up except membrane filtration. The LOQs were < 1 mg/kg, but no further details were disclosed. Vanerková et al. (2014) also report in a conference abstract on the development of an LC-MS/MS method for lupanine, lupinine and sparteine in lupin seeds with LOQs of 2.6, 3.3 and 0.14 mg/kg, respectively.

**Analytical standards, certified reference materials and proficiency testing schemes**

The availability of commercial QA reference standards is limited. Lupanine, lupinine and sparteine are available from a number of different suppliers, but the other QAs are either available from a single source only or not available at all. Isotopically labelled analogues are also not available. No certified materials are commercially available and no proficiency testing schemes for testing of QAs in lupin seeds or lupin products have been identified.

### 1.3.3. Botanical origin, varieties and cultivation

The genus *Lupinus* belongs to the family of Fabaceae (Leguminosae), a large family of plants, to which also important food and feed crops such as beans (e.g. fava beans (*Vicia faba*), peas (*Pisum sativum*), lentils (*Lens culinaris*), peanuts (*Arachis hypogaea*) and soybeans (*Glycine max*) belong. Of these food crops only lupins are known to produce QAs (Wink, 2003). Within the Fabaceae family, the genus *Lupinus* is placed in the tribe Genisteeae, which comprises also the brooms (a general term describing species of the genera *Cytsis*, *Genista*, *Spartium*) and *Laburnum* (golden rain). Almost all species of the Genisteeae tribe produce QAs (Wink and Mohamed, 2003; Boschin and Resta, 2013). An estimated 250-500 different lupin species have been described in the literature, the majority of them

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2 https://www.sisweb.com/software/ms/nist.htm [accessed in December 2018].
found only in North or South America (so called New World lupin species). In Europe and North Africa, only 12 species occur (so-called Old-World lupin species) (Wink et al., 1995; Boschin and Resta, 2013). Lupin seeds are rich in protein which makes them an interesting alternative crop for e.g. soybeans. Worldwide, four lupin species are cultivated on a commercial scale for food and feed purposes: *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* (Gresta et al., 2010; Boschin and Resta, 2013; Carvajal-Larenas et al., 2016; Magalhaes et al., 2017).

The main use of lupin seeds globally is as a high protein feed for livestock, where it is used as an alternative to oilseed meals, while only approximately 4% is grown for food (Lawrance, 2007). Furthermore, lupin seeds represent a small proportion of the total amount of feed used for livestock. In 2016/17, EU-grown lupin seeds accounted for approximately 5% of the EU produced high-protein oilseeds (soybean meal, rapeseed meal and sunflower meal) and less than 2% of the oilseeds (EU produced and imported) used in the manufacture of compound feeds.\(^3\)

In Figure 2, the production of lupin seeds in countries of the European Union (EU) over the period 2002–2016 is presented. Data are available from the FAOSTAT database,\(^4\) but these are not specified for the different lupin species. In the EU, Poland has emerged in recent years as the main producer of lupin seeds, with a current annual production above 200,000 tonnes. Germany (ca. 50,000 tonnes/year) and France (ca. 16,000 tonnes/year) are also important producers.

![Figure 2: Lupin seed production in EU countries in the period 2002–2016 (based on data extracted from FAOSTAT)](image)

Globally, since the mid-1980s Australia is the biggest producer of lupin seeds, mostly *L. angustifolius* (Figure 3). In the last decade of the 20th century, Australia dominated the global market with a market share of over 90% and an annual production of more than 1 million tonnes (FAOSTAT; Lawrance, 2007; Sweetingham and Kingwell, 2008). Since then, production has steadily declined to around 50% of the world production. In recent years, the Russian Federation has become an important producer of lupin seeds, mostly *L. luteus* (Sweetingham and Kingwell, 2008). Because the large-scale production of lupin seeds is concentrated in only a few countries, the global yearly production can fluctuate significantly, due to a strong dependence on the prevailing weather conditions (Lawrance, 2007; Sweetingham and Kingwell, 2008; OGTR, 2013).

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\(^3\) Source: The compound Feed Industry in the EU Livestock Economy (FEFAC.eu).

\(^4\) [http://www.fao.org/faostat/en/#home](http://www.fao.org/faostat/en/#home). The FAOSTAT database provides free access to food and agriculture data for over 245 countries and territories and covers all FAO regional groupings from 1961 to the most recent year available.
Wild lupin species contain high amounts of the bitter tasting QAs. Nevertheless, in the Mediterranean region the origins of domestication of *L. albus* can be traced back to 4,000 years ago (Kurlovich, 2002). Traditionally, the levels of QAs in the seeds have been substantially reduced by soaking the beans in salted water and washing. In the 20th century, breeding efforts have resulted in the successful introduction of low-alkaloid containing varieties, so called ‘sweet lupins’ (Hondelmann, 1984; Muzquiz et al., 1994; Frick et al., 2017). Often, a discrimination between sweet (less than 500 mg/kg total QAs) and bitter varieties (more than 10,000 mg/kg total QAs) is made (Pilegaard and Gry, 2008). However, semi-sweet and semi-bitter varieties are also common (Boschin et al., 2008).

The EU database on registered plant varieties currently lists 23 varieties of *L. albus*, 50 varieties of *L. angustifolius* and 14 varieties of *L. luteus*. *L. albus* is grown predominantly in countries of the Mediterranean region, *L. angustifolius* is produced primarily in Eastern Europe and Germany while *L. luteus* is mainly cultivated in Poland. *L. mutabilis* is not grown on a commercial basis in the EU (Magalhães et al., 2017).

In Appendix C, the biosynthetic pathway towards the formation of QAs is shown. QAs are derived from the amino acid lysine that is converted into cadaverine by the enzyme lysine decarboxylase (LDC). From two molecules of cadaverine, after a cascade of steps, the bicyclic (-)-lupinine is formed, while by combination of three molecules of cadaverine, via an intermediate diiminium cation, the tetracyclic QAs such as (-)-sparteine, (+)-lupanine and (-)-multiflorine are formed (Golebiewski and Spenser, 1988). Further diversification can occur via hydroxylation and esterification (Bunsupa et al., 2013). The palette of QAs produced is often specific for the individual lupin species (Wink et al., 1995).

Appendix D, Tables D.1–D.4 give an overview of levels of QAs in seeds of the species *L. albus*, *L. luteus*, *L. angustifolius* and *L. mutabilis*. The results shown in Appendix D and summarised in Table 1 are from papers in which the chemical analysis is carried out by GC or HPLC. As seen in Table 1, lupanine is in general the most abundant QA in *L. albus*, *L. angustifolius* and *L. mutabilis*. In *L. luteus* lupinine is the most common QA together with sparteine. Lupinine is only found in *L. luteus* while sparteine is also found in the three other species but in *L. albus* and *L. angustifolius* only in minor amounts. As it can be seen from Table 1, the amount of the QAs can differ considerably within the same species. In addition, the total concentration of QAs as well as the distribution and the concentration of the individual QAs from the same varieties change with growth place and year. For example, the total concentration of the cultivar Multitalia (*L. albus*) differs from 2,448 mg/kg to 6,292 mg/kg when the seeds are grown in two different growing seasons at two different places in Italy (Annicchiarico et al., 2014; see Appendix D, Table D.1).

The occurrence of QAs in the four relevant lupin species has also been reviewed by the Nordic Council of Ministers in 2008 (Pilegaard and Gry, 2008), by Carvalja-Larena in 2016 and most recently by the Bundesinstitut für Risikobewertung/German Federal Institute for Risk Assessment (BfR) in 2017.
Appendix D, Table D.5 summarises the outcome of these reviews. The results shown in Table D.5 support the results shown in Table 1, since also here lupanine is in general the most commonly found QA in *L. albus*, *L. angustifolius* and *L. mutabilis* while for *L. luteus* it is lupinine and sparteine. In addition, the results in Table D.5 show that the contribution of the same QA can differ considerably within the same species.

**Table 1:** Summary of the occurrence of quinolizidine alkaloids in seeds of the species *L. albus*, *L. luteus*, *L. angustifolius* and *L. mutabilis*

| Variety(a) | Total amount (mg/kg) | Quinolizidine alkaloids identified | Contribution of individual quinolizidine alkaloids to the total amount(b),(d) |
|------------|-----------------------|------------------------------------|------------------------------------------------------------------------------|
| **L. albus** |                       |                                     |                                                                              |
| Bitter     | < 1,000–23,600        | Lupanine                           | 58.92% 0.63–19% 7.6–11% 1.1% 0.30–1.0% 0.68% 0.13% 0.08%                     |
|            |                       | Albine                             |                                                                              |
|            |                       | Multiflorine                       |                                                                              |
|            |                       | Angustifoline                       |                                                                              |
|            |                       | α-Isolupanine                      |                                                                              |
|            |                       | 13α-Oh-Lupanine                    |                                                                              |
|            |                       | Sparteine                          |                                                                              |
|            |                       | 13α-Tigloyloxylupanine             |                                                                              |
| Bitter     | 14,516                | Lupanine                           | 11,219 mg/kg 1,012 mg/kg 492 mg/kg 268 mg/kg 102 mg/kg 24 mg/kg              |
|            |                       | Albine                             |                                                                              |
|            |                       | 13α-Oh-Lupanine                    |                                                                              |
|            |                       | Angustifoline                       |                                                                              |
|            |                       | 13α-Tigloyloxylupanine             |                                                                              |
|            |                       | Tetrahydrorhombifoline             |                                                                              |
| Sweet      | 50–1,656              | Lupanine                           | 26–1,378 mg/kg 3–157 mg/kg Not detected–88 mg/kg 10–162 mg/kg Not detected–65 mg/kg Not detected–44 mg/kg Not detected–22 mg/kg 1.0–13 mg/kg |
|            |                       | 13α-Oh-Lupanine                    |                                                                              |
|            |                       | 13α-Angeloxyloxylupanine           |                                                                              |
|            |                       | Albine                             |                                                                              |
|            |                       | Angustifoline                       |                                                                              |
|            |                       | 13α-Tigloyloxylupanine             |                                                                              |
|            |                       | α-Isolupanine                      |                                                                              |
|            |                       | Tetrahydrorhombifoline             |                                                                              |
| Not stated | 146–49,100            | Lupanine                           | 28–77% 2.4–33% < 1–23% 1–22% 0.02–16% 0.29–10% 8.9% < 1% < 1%                  |
|            |                       | 13α-Oh-Lupanine                    |                                                                              |
|            |                       | 13α-Angeloxyloxylupanine           |                                                                              |
|            |                       | Multiflorine                       |                                                                              |
|            |                       | Albine                             |                                                                              |
|            |                       | Angustifoline                       |                                                                              |
|            |                       | 13α-Tigloyloxylupanine             |                                                                              |
|            |                       | α-Isolupanine                      |                                                                              |
|            |                       | Sparteine                          |                                                                              |
| Not stated | 38–52,380             | Lupanine                           | 17–46,240 mg/kg Not detected–4,710 mg/kg 10–2,596 mg/kg Not detected–750 mg/kg Not detected–616 mg/kg Not detected–348 mg/kg Not detected–250 mg/kg Not detected–112 mg/kg Not quantified–50 mg/kg 6.5–44 mg/kg 2.1 mg/kg |
|            |                       | 13α-Oh-Lupanine                    |                                                                              |
|            |                       | Albine                             |                                                                              |
|            |                       | Angustifoline                       |                                                                              |
|            |                       | Multiflorine                       |                                                                              |
|            |                       | 13α-Tigloyloxylupanine             |                                                                              |
|            |                       | α-Isolupanine                      |                                                                              |
|            |                       | 13α-Angeloxyloxylupanine           |                                                                              |
|            |                       | Lupinine                           |                                                                              |
|            |                       | Tetrahydrorhombifoline             |                                                                              |
|            |                       | Sparteine                          |                                                                              |
| Variety<sup>(a)</sup> | Total amount (mg/kg) | Quinolizidine alkaloids identified | Contribution of individual quinolizidine alkaloids to the total amount<sup>(b),(d)</sup> |
|----------------------|-----------------------|-----------------------------------|-----------------------------------------------|
| **L. angustifolius**  |                       |                                   |                                               |
| Bitter               | 20,600                | Lupanine 13α-OH-Lupanine, Angustifoline, Tetrahydrorhombifoline, α-Isolupanine, Sparteine | 71%, 17%, 5.8%, 2.8%, 1.2%, 0.1% |
| Bitter               | 10,800–19,800         | Lupanine 13α-OH-Lupanine, α-Isolupanine | 4,000–12,900 mg/kg, 5,000–6,600 mg/kg, 213–237 mg/kg |
| Sweet                | 840–1,800             | Lupanine 13α-OH-Lupanine, α-Isolupanine, Sparteine, Angustifoline, Tetrahydrorhombifoline | 4.0–50%, 19–21%, 6.4–14%, 2.5–4.8%, 2.7–4.4%, 0.0–1.0% |
| Sweet                | 44–2,120              | 13α-OH-Lupanine, Lupanine, Angustifoline, α-Isolupanine | 18–943 mg/kg, 18–862 mg/kg, 7–226 mg/kg, 8–90 mg/kg |
| Not stated           | 652–19,176            | Lupanine 13α-OH-Lupanine, Angustifoline, α-Isolupanine, Multiflorine, Tetrahydrorhombifoline, Sparteine | 36–70%, 12–37%, 10–30%, <1–12%, 0.95%, <1%, <1% |
| Not stated           | 15–24,950             | Lupanine 13α-OH-Lupanine, Angustifoline, α-Isolupanine, 13α-OH-Lupanine, Sparteine | 7.4–20,570 mg/kg, <LOQ–3,830 mg/kg, 40–550 mg/kg, 5.6–26 mg/kg, <LOQ–10 mg/kg |
| **L. luteus**        |                       |                                   |                                               |
| Sweet                | 389–895               | Sparteine, Lupinine, Lupanine     | 2–234 mg/kg, 3–281 mg/kg, 6.4–37 mg/kg        |
| Not stated           | 1,020<sup>(c)</sup>   | Lupinine, Sparteine, 13α-OH-Lupanine, Lupanine, Tetrahydrorhombifoline | 27–60%, 30–56%, 6.3%, <1–2.9%, <1% |
| Not stated           | 9–10,630              | Sparteine, Lupinine, 13α-OH-Lupanine, Lupanine | 7.1–7,890 mg/kg, 0–2,420 mg/kg, <LOQ–10 mg/kg, <LOQ–0.5 mg/kg |
| **L. mutabilis**     |                       |                                   |                                               |
| Not stated           | 31,000<sup>(e)</sup>  | Lupanine, Sparteine, 13α-OH-Lupanine, Tetrahydrorhombifoline, 13α-Angeloyloxylupanine, Angustifoline, 13α-Tigloyloxylupanine, α-Isolupanine, Multiflorine | 47–58%, 7.4–16%, 7–15%, 2–3.5%, 1.6–2%, 1%, 0.3–1%, 0.3–7%, 0.14–<1% |

LOQ: limit of quantification.
(a): As stated in the literature.
Quinolizidine alkaloids in feed and food

1.3.4. Previous risk assessments

No previous animal health risk assessments on QAs, lupins or lupin derived feed have been identified by the CONTAM Panel.

In 1996, the UK Advisory Committee on Novel Food and Processes has published a report on seeds from *L. angustifolius* (FSA, 1996) in which they, considering advice from the Food Advisory Committee and the Committee on Toxicity (COT), concluded that seeds and products from *L. angustifolius* are safe for use provided that an alkaloid level of 200 mg/kg is not exceeded in seeds or products thereof as any anticipated human exposure would be significantly lower than the NOAEL of 1,000 mg lupin alkaloids/kg diet identified by the Committee on Toxicity of Chemicals in Food.

In 1998, the French Directorate General of Health has issued a favourable opinion regarding the use of flour from *L. albus* var. ARES in food, provided that the alkaloid levels in such flour remain below 200 mg/kg (Direction générale de la santé, 1998).

In 2001, the Australia New Zealand Food Authority (ANZFA), the former Food Standards Australia New Zealand (FSANZ), published a risk assessment on lupin alkaloids in food (ANZFA, 2001). It was concluded that the no-observed-effect level (NOEL) of 90–105 mg/kg body weight (bw) per day for lupin alkaloids of *L. angustifolius* and *L. luteus* obtained in subchronic rat studies (Ballester et al., 1980; Butler et al., 1996; Robbins et al., 1996) is about three times higher than the lethal dose of about 30 mg/kg bw seen in human poisoning cases with lupin alkaloids where sparteine was the major alkaloid. It was suggested that the rat is not appropriate to derive tolerable exposure levels in humans. Thus, ANZFA derived a provisional tolerable daily intake (PTDI) of 35 μg/kg bw per day based on reports of lupin consumption which indicate that in humans daily doses of 0.35 mg lupin alkaloids/kg bw do not cause adverse effects, and adding an uncertainty factor (UF) of 10 to account for intra-individual variations. Dietary exposure was estimated assuming that 5% of all flour-based products consumed contained 10% lupin flour and assuming an alkaloid level of 130 mg alkaloids per kg for lupin seeds intended for human consumption (Robbins et al., 1996). Based on a P95 flour consumption of 244.8 g/day and a body weight of 70 kg, ANZFA concluded that this leads to a 95th percentile dietary exposure of 2 μg lupin alkaloids/kg bw per day which is well below the PTDI of 35 μg/kg bw per day.

In 2008, the Nordic Council of Ministers issued a toxicological review and recommendations on alkaloids in edible lupin seeds (Pilegaard and Gry, 2008). They noted that humans, and especially children, seem to be more sensitive towards lupin alkaloids than rodents. The lethal dose in children was estimated to be 11–25 mg/kg bw, while oral rat LD50s range from 1,700 to 2,300 mg/kg bw. As a consequence, they concluded that the results available from several repeated dose studies with rats are of limited value. They also noted that several non-edible lupin species have been associated with developmental effects in livestock animals. The QA anagyrine is believed to cause 'crooked calf disease' in offspring of cows that consume lupins. Consumption of *Lupinus consentinii* is associated with developmental effects in lambs and calves. Its major QA is multiflorine, according to the authors 'structurally related' to anagyrine and occurring in edible lupins in amounts of up to 18% of the total alkaloid amount. In the absence of consumption data, daily intake levels of alkaloids were calculated for adults and children assuming that adults (60 kg bw) eat 300 g bread and 100 g pasta each containing 15% lupin flour and in addition 50 g lupin seeds as a snack. For children (20 kg bw), it was assumed that they eat 150 g bread, 75 g pasta and 30 g lupin seeds. In a first scenario, a concentration of 200 mg alkaloids/kg lupin seeds was used and in a second scenario 500 mg/kg. In a third scenario, it was assumed that only lupin seed snacks containing 500 mg alkaloids/kg are consumed. Resulting daily intakes for adults and children were 0.32 and 0.57 mg/kg bw (first scenario), 0.79 and 1.4 mg/kg bw (second scenario) and 0.42 and 0.75 mg/kg bw (third scenario), respectively.

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6 https://cot.food.gov.uk/sites/default/files/cot/cotannualreport1995.pdf
7 The disease is characterized by twisted or bowed limbs (arthrogryposis), twisted or bowed spine (scoliosis or kyphosis), twisted neck (torticollis), cleft palate, or a combination of any of them.
The BfR has recently assessed human health risks from the intake of QAs, such as lupanine, lupinine, and sparteine, as components of lupin seeds derived from various lupin species, e.g. *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* (BfR, 2017a,b). Based on the available data, the BfR considered lupanine, lupinine and sparteine to act via a similar mode of action and likely in an additive way, sparteine showing the highest potency. The limited information available on the dose effect relationships, did not allow to identify a NOAEL for humans. Altogether, the analysis of the reported cases revealed that intoxication has been repeatedly attributed to the insufficient debittering of lupin seeds by consumers. Since consumption data on foods in Germany that contain lupin seeds or a processed form thereof (e.g. lupin flour) were lacking, a representative ‘Consumer survey on the consumption of lupin seeds’ commissioned by the BfR was conducted throughout Germany in 2016. The proportion of consumers who had consciously eaten lupin seed-based food was small (9.2% of respondents). For the acute exposure estimation, the BfR assumed a total QA concentration of 200 mg/kg lupin seeds. The highest acute dietary QA exposure was estimated for ‘Lupini beans as a snack’ (0.286 mg/kg bw per day) and ‘Patties’ (0.229 mg/kg bw per day). Estimated acute total QA intake via foods from the remaining categories ranged from 0.003 to 0.057 mg/kg bw per day. Estimates for chronic total QA intake were calculated based on an assumed total QA concentration of 60 mg/kg seeds and were the same for consumers of ‘Lupini beans as a snack’ and ‘Patties’ with a median of 0.013 mg/kg bw per day. When assessing the risks, BfR concluded that 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 (HGBVs). For the assessment of acute risks, BfR compared total intakes of QAs to the pharmacological threshold dose of sparteine of 0.2 mg/kg bw reported by Blaschek et al. (2006) and Thies (1986), which was chosen as a reference point. BfR concluded that the margin of exposure (MOE) to this reference point should be more than 1 to account for the uncertainty in the data base, including higher sensitivities of special population groups, such as pregnant women and poor metabolisers (PMs). Thus, BfR considered the MOE of 1, which resulted for the two above mentioned food categories of ‘Lupini beans as a snack’ and ‘Patties’, as inadequate. For the remaining food categories, the MOE lay between 4 and 70. With respect to chronic risks, BfR noted, that current knowledge did not allow deriving a toxicological reference point.

### 1.3.5. Legislation

In this opinion, where reference is made to Regulations, the reference should be understood as relating to the most recent amendment, unless otherwise stated.

Article 2 of Council Regulation (EEC) No 315/93

8 stipulates that food containing a contaminant in an amount unacceptable for public health shall not be placed on the market, that contaminant levels should be kept as low as can reasonably be achieved and that, if necessary, the European Commission may establish maximum levels for specific contaminants. These maximum levels (MLs) are laid down in the Annex of Commission Regulation (EC) No 1881/2006

9 and may include MLs for the same contaminants in different foods, analytical detection limits and reference to the sampling and analysis methods to be used. Currently, no MLs are set for QAs in food.

Council Directive 2002/32/EC

10 regulates undesirable substances in products intended for animal feed. Annex I to this regulation contains a list with MLs for certain inorganic and organic feed contaminants. QAs are currently not considered in this Directive. Only sweet lupins (SLs) can be used for animal feeding as specified in the Catalogue of feed materials. Commission Regulation (EC) No 1121/2009 of 29 October 2009

12 defines SLs as ‘those varieties of lupins producing seed comprising not more than 5% bitter seeds. The bitter seed content shall be calculated in accordance with the test set out in Annex II to this Regulation.’

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8 Council Regulation (EEC) No 315/93 of February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1–5.

9 Regulation (EC) No 1881/2006 of the European Parliament and the Council of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

10 Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10–21.

11 Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29 30.1.2013, p. 1–64.

12 Commission Regulation (EC) No 1121/2009 of 29 October 2009 laying down detailed rules for the application of Council Regulation (EC) No 73/2009 as regards the support schemes for farmers provided for in Titles IV and V thereof. OJ L 316, 2.12.2009, p. 27–64.
The Novel Food Catalogue\textsuperscript{13} contains products of animal and plant origin subject to the Novel Food Regulation (EU) 2015/2283\textsuperscript{14} based on information provided by EU Member States. It serves as an orientation whether or not certain products need to be authorised under the Regulation. \textit{L. albus}, \textit{L. angustifolius} and \textit{L. luteus} are included in the catalogue but neither of them requires authorisation as they have been marketed prior to 15 May 1997.

In Australia and New Zealand, an ML of 200 mg lupin alkaloids/kg apply for lupin flour, lupin kernel flour, lupin kernel meal and lupin hulls and ML of 5 mg/kg for sparteine in alcoholic beverages.\textsuperscript{15}

2. Data and methodologies

2.1. Methodology for data collection and study appraisal

A call for an ‘Extensive literature search and selection for relevance of studies related to the chemistry and toxicity of glycoalkaloids and QAs in food and feed’ aiming at identifying and evaluating literature related to the present assignment (and to another mandate of the CONTAM Panel on glycoalkaloids which is not further considered here). The call was launched as a Reopening competition for a specific contract under multiple framework contract CT/EFSA/AMU/2014/01 Lot 2. The University of Chemistry and Technology Prague was awarded with the contract and a final project report has been delivered in November 2017 and was published together with the present opinion (University of Chemistry and Technology Prague, 2019). Briefly, following standardised protocols for identification and evaluation (as described in University of Chemistry and Technology Prague, 2019) potentially relevant studies/reviews issued in the period from 1950 to 2017 (the search was carried out on 29 June 2017) were identified and the numbers of publications were as follows per scientific area. Analytics/chemistry determination of chemical identity, formation: 1,021; Toxicokinetics: 103; Mode of action: 88; \textit{In vivo} toxicity: 60; \textit{In vitro} toxicity: 15; Observations in humans: 13; Adverse effects in farm animals, horses and companion animals: 61; Food occurrence: 1,042; Feed occurrence: 1,042 and factors in influencing levels of QAs: 180. The total number of papers identified was 1,768. A series of publications were identified as relevant for more than one scientific area. The abstracts of these publications were then again screened by the Working Group members, and a total of 175 publications were considered as potentially relevant for the assessment and the originals were retrieved. Furthermore, a search was done to identify papers that were published after the search was carried out or that were not captured in the search above (see Appendix E for further details).

In addition, a ‘snowballing’ approach\textsuperscript{16} was applied (see Jalali and Wohlin, 2012) in order to obtain any additional relevant information not captured in the systematic research.

The publications were in the assessment if considered as relevant by applying expert judgement. It should be noted that lupin species other than \textit{L. albus}, \textit{L. angustifolius}, \textit{L. luteus} and \textit{L. mutabilis} also contain QAs that are relevant for the current assessment, therefore also studies with \textit{L. caudatus}, \textit{L. leucophyllus}, \textit{L. argenteus}, \textit{L. montanus}, \textit{L. exaltatus} and \textit{L. mexicanus} were considered for the hazard identification and characterisation.

In addition, the draft opinion underwent a public consultation from 23 May 2019 to 5 July 2019. The comments received and how they were taken into account when finalising the scientific opinion were published in an EFSA Technical Report (EFSA, 2019). During this public consultation additional information was submitted to EFSA (see Documentation provided to EFSA). Following a comment submitted during the public consultation, an additional literature search was conducted in Scifinder.\textsuperscript{17}

Following screening of title and abstract, 60 references were retrieved for screening of the full text.

\textsuperscript{13} http://ec.europa.eu/food/safety/novel_food/catalogue/search/public/index.cfm
\textsuperscript{14} Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1–22.
\textsuperscript{15} Australia New Zealand Food Standards Code – Schedule 19: Maximum levels of contaminants and natural toxicants. Registered 18 April 2017. Id F2017C00333. https://www.legislation.gov.au/Series/F2015L00454
\textsuperscript{16} Identifying articles that have been cited in articles found in a search.
\textsuperscript{17} Search string: Substance Identifier “sparteine” > substances (1) > get references (1543) > refine “1960-1980”. Results: 408.
2.2. Food and feed occurrence data submitted to EFSA

2.2.1. Data collection

Following a mandate from the European Commission, occurrence data on QAs were acquired via the call for collection of occurrence data on chemical contaminants in food and feed, issued by the DATA Unit. European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on QAs in food and feed. The data submission followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a) using the data model 'Standard sample description' (SSD1 or SSD2) (EFSA, 2010a, 2013). At the moment of data extraction, a total of 1,465 analytical results on 13α-OH-lupanine, 13α-angeloyloxylupanine, 13α-tigloyloxylupanine, albine, angustifoline, α-isolupanine, lupanine, lupinine, multiflorine, sparteine, tetrahydrorhombifoline in feed (n = 390) and food (n = 1,075) were available in the EFSA Chemical Occurrence database.

2.2.2. Data validation and analysis

Chemical occurrence data were managed following the EFSA standard operating procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data' to guarantee an appropriate quality of the data used in the exposure assessment. Special attention was devoted to the identification of duplicates and to different parameters such as 'Sampling strategy', 'Sampling method', 'Sampling year', 'Sampling country', 'Analytical methods', 'Reporting unit', 'LOD/LOQ', and to the correctness of the food classification codification of the different samples under FoodEx2 classification system (see Section 2.3.3). In the analysis of QA occurrence data, the left-censored data (results below LOD or below LOQ) were treated by the substitution method detailed in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The lower bound (LB) is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD or LOQ. The upper bound (UB) is obtained by assigning the numerical value of the LOD to values reported as higher than the LOD and the LOQ to values reported as higher than the LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

In this Scientific Opinion, the ‘Total QA’ (TotQA) concentration was calculated by summing the concentrations (expressed as mg/kg) of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine and α-isolupanine. For calculation of the UB TotQA, the LOD or LOQ was used for each substance for which a left-censored result was reported.

2.3. Food and feed consumption data

2.3.1. Food consumption

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level. Details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011). The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. The latest version of the Comprehensive Database updated in 2018 contains results from a total of 60 different dietary surveys carried out in 25 different Member States covering 119,458 individuals. The age classes considered are the following:

- Infants: < 12 months old
- Toddlers: ≥ 12 months to < 36 months old
- Other children: ≥ 36 months to < 10 years old
- Adolescents: ≥ 10 years to < 18 years old
- Adults: ≥ 18 years to < 65 years old
- Elderly: ≥ 65 years to < 75 years old
- Very elderly: ≥ 75 years old.

Four additional surveys provided information on specific population groups: ‘Pregnant women’ (≥ 15 to ≤ 45 years old for Latvia; 17 to 46 years for Portugal) and ‘Lactating women’ (≥ 28 to ≤ 39 years for

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18 On 3/1/2019.
Greece; 18 years to 45 years for Estonia). When for one country and age class two different dietary surveys were available, only the most recent one was used. Dietary surveys and the number of subjects available for acute exposure assessment to QAs (43 surveys from 25 countries) are described in Table A.1 of Annex A. Consumption data were collected using single or repeated 24-h or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading. Detailed information on the different dietary surveys available in the Comprehensive Database can be found on the dedicated page of the EFSA website (http://www.efsa.europa.eu/en/food-consumption/comprehensive-database).

2.3.2. Feed consumption

Lupin seeds may be fed as the whole seed or ground, and incorporated with other feed materials in manufactured compound feeds (as complete or complementary feedingstuffs). Lupin seeds and meal are used predominantly in ruminant diets as a source of protein (typically 30–40% depending on the variety of lupin). Their use in the diets of single-stomached animals has been limited mainly due to the lower concentrations of certain essential amino acids when compared with other protein sources such as soybean meal. For poultry, high inclusion rates tend to be restricted to avoid problems associated with excess moisture in the excreta (resulting in 'wet-sticky droppings') due to the high levels of lupin non-starch polysaccharides. SLs are used in aquaculture, where the seeds are usually dehulled to improve digestibility.

Details of the assumed feed intakes and live weights for different farmed animals, horses and companion animals have been obtained from a number of different sources, details of which are given in Appendix G.

2.3.3. Food classification

Consumption and occurrence data were classified according to the ‘Exposure hierarchy’ of the FoodEx2 classification system (EFSA, 2015). This is based on a food list of 2,673 entries, referred as ‘basic FoodEx2 code’, aggregated into food groups and broader food categories in a hierarchical parent-child relationship (up to 7 levels). In addition, a catalogue of 28 ‘facets’ is available in order to describe further characteristics of the foods, such as physical state (e.g. powder, liquid, etc.) or processing technology (e.g. grinding, milling, crushing, etc.).

2.3.4. Feed classification

Feed samples were as well classified according to the ‘Feed hierarchy’ of the FoodEx2 system (EFSA, 2015). This is based on the catalogue of feed materials specified in the Commission Regulation (EU) No 68/2013 and the feed classification provided by Regulation (EU) No 575/2011 (and following modifications).

2.4. Methodology for Exposure assessment

2.4.1. Human exposure assessment

The CONTAM Panel decided to assess acute exposure to TotQAs for consumers of lupins seeds or lupin-based foods according to four scenarios that are detailed in Section 3.3.1. For this aim, food consumption data from the EFSA Comprehensive Database were extracted for the following FoodEx2 categories: 'Lupins seeds without pods' and 'Meat imitates', as a proxy of the consumption of lupins seeds or lupin-based foods. Average and high (P95) exposure was calculated for different countries and population groups, when the number of consumption days was sufficient to allow the calculations of reliable statistics (details in Section 3.3.1). Acute exposure was assessed independently for each food category, the daily consumption amount (standardised by the individual's body weight) was multiplied for the highest reliable percentile of the corresponding occurrence data. When the number of samples was too small to allow the calculation of a percentile (below 11 observations), the average occurrence level was used. Occurrence data were considered under the LB and UB approach, when there was no difference only one approach was shown. All analyses were run using the SAS Statistical Software (SAS enterprise guide 5.1).
2.4.2. Animal exposure assessment

Ideally, animal exposure assessment would be based on levels of QAs in species-specific compound or complementary feeds, together with their assumed levels of intake. However, no data on QA levels in compound/complementary feeds were available, and therefore exposures have been estimated based on levels of QAs in the seeds and recommended levels of inclusion in the diets of farm livestock, details of which are given in Appendix G.

According to EFSA (2011), caution is needed when calculating acute exposure (95th percentile) where data on less than 60 samples are available, since the results may not be statistically robust. Due to the limited database, it has not been possible to estimate 95th percentile exposures.

2.5. Methodology for Risk characterisation

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by WHO (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by WHO (2009), EFSA guidance pertaining to risk assessment has been applied for the present assessment. In brief, the EFSA guidance covers the procedures currently used within EFSA for the assessment of dietary exposure to different chemical substances and the uncertainties arising from such assessments. For details on the specific EFSA guidance applied see Appendix F.

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics and metabolism

3.1.1.1. Laboratory animals and humans

Except for sparteine, there is little or no information on the toxicokinetics and metabolism of QAs that may occur in feed and food. Sparteine, as sparteine sulfate, was used as a drug to treat cardiac arrhythmias and induce uterine contractions in labour, and as a phenotypic marker of cytochrome P450 (CYP) 2D6 drug metabolism activity (Thies, 1986).

**Sparteine**

Sparteine elimination was studied in rats following intra-arterial or portal venous administration of sparteine sulfate (50 mg/kg bw equivalent to 27.7 mg/kg bw sparteine) (Van der Graaff et al., 1986). In rats with bile fistula, no sparteine was recovered in the bile collected during 3 h, and about 25% of the dose was present as a metabolite of sparteine, of which the identity could not be established. Further studies in rats showed that sparteine was oxidised to lupanine as verified by the chromatographic retention time and the mass spectrum similar to a standard (Chaudhuri and Keller, 1990). At a dose of 50 mg/kg bw sparteine intraperitoneally (i.p.), both lupanine and 2,3-dehydrosparteine were observed in rat urine, whereas following sparteine in doses up to 16.8 mg/kg bw only lupanine was detectable in urine. At the latter dose of sparteine, pretreatment of the rats with the CYP inhibitor SKF 525A reduced the urinary lupanine substantially indicating a role of CYP in the formation of lupanine. Pretreatment with disulfiram, an inhibitor of aldehyde dehydrogenase, a cytosolic enzyme, and CYP2E1 (Emery et al., 1999), also inhibited lupanine formation indicating that conversion of sparteine to lupanine could take place via an intermediate, and likely was involving both microsomal and cytosolic enzymes. The conversion of sparteine to lupanine did not occur in *in vitro* studies using rat liver microsomes or 9,000g supernatant (S9).

In humans, orally administered sparteine sulfate (200 mg corresponding to 110.8 mg of sparteine base, assuming that sparteine sulfate is used in the form of the pentahydrate) was rapidly absorbed (about 70%) with a maximum concentration in plasma after about 45 min (Dengler et al., 1970). Dengler and co-workers (1970) also used rats and found that sparteine rapidly distributed to most tissues.

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19 In cases where the stereochemical configuration of sparteine is not indicated in the text, it was not defined in the underlying reference. With regard to the antiarrhythmic drug Depasan®, the active ingredient was defined as (−)-sparteine sulfate (Rote Liste, 1983).

20 According to Milne (2018) and The Merck Index (2006), it is assumed that sparteine sulfate is used in the form of the pentahydrate (MW: 422.54). A dose of 50 mg sparteine sulfate pentahydrate/kg bw would then correspond to a dose of 27.7 mg sparteine/kg bw (MW: 234.38).
organs reaching much higher concentrations in comparison with plasma, particularly, in lungs, adrenals, spleen and kidney. Elimination took place with a half-life in plasma of about 2 h in both rats and humans. Following intravenous injection to humans (200 mg of sparteine sulfate) about 35% of the dose was recovered unchanged in urine during 24 h.

In humans, sparteine is oxidised by CYP2D6 to two metabolites, 2,3-dehydrosparteine, being the major metabolite, and 5,6-dehydrosparteine, a minor metabolite (Eichelbaum et al., 1986). Results from extensive experiments are in agreement with the hypothesis that the oxidation at least at the 2,3-position proceeds by direct carbon oxidation and not via N1-oxidation as was first suggested (Eichelbaum et al., 1979a; Guengerich, 1984; Ebner et al., 1996). There are several polymorphisms in CYP2D6 and two distinct phenotypes have been identified: PMs of sparteine (5–10% of Caucasians) and extensive metabolisers (EMs). In addition, a subgroup of the latter have been identified as ultra-rapid metabolisers of sparteine (about < 1–2% in the Scandinavian populations, but a larger fraction of the Spanish (8%) and Ethiopian (29%) populations) (Bathum et al., 1998). The PMs phenotype has a strong impact on the elimination of sparteine as in this group elimination takes place as unchanged compound entirely via a low capacity renal excretion. In contrast, the elimination proceeds largely via metabolites in EMs. The resulting plasma clearance in PMs decreased to one-third and the plasma half-life increased from about 2.5 h in EMs to about 6.8 h in PMs (Eichelbaum et al., 1979b). As unchanged sparteine and conversion to the two dehydrosparteine metabolites could only account for 60% of the dose in urine (Eichelbaum et al., 1979a,b), suggestions have been made for the missing dose of sparteine: formation of other metabolites, and further metabolism or degradation of the dehydrosparteine metabolites. There are no reports that humans may convert sparteine into lupanine even if this is a major metabolite in the rat. There is no information on whether conjugated metabolites might be formed from sparteine and its metabolites.

Lupanine

Wittenburg and Nehring (1965) conducted two experiments with Wistar rats, which were given lupanine as lupanine hydrochloride in the feed. In a first experiment, 3.4 mg lupanine/g feed (dry weight (dw)) was given as a single portion of feed (23.2–33.1 mg/rat; bw not specified). About 60% of the consumed dose was excreted in urine during day one. In a second experiment lasting for 29 days, rats were administered lupanine in feed in increasing concentrations (4 days 1, 4 days 3, 4 days 6 and 4 days 9 mg/g feed (dw)). In both experiments, between 70 and 80% of the amount of lupanine consumed was excreted, 50–70% in urine and 10–14% in faeces. About half of the total amount was excreted unchanged and the rest as OH-lupanine21 (30–40% of the lupanine intake). No accumulation of lupanine in the rats took place.

The disposition of lupanine and 13α-OH-lupanine were investigated in two groups of humans, namely CYP2D6 PM (N = 4) and EMs (N = 7) (Petterson et al., 1994). The compounds (10 mg) were given in gelatine capsules followed by 700 mL of water to fasting participants. The design was a randomised cross-over test with a 2 weeks wash-out period between the two compounds. The ingested QAs were rapidly excreted into urine with total recoveries at 72 h ranging from 89% to 103%, and it did not differ between PM and EM groups. The half-lives in urine were on average about 6 h, ranging from 3 to 11 h for both compounds and both groups. Lupanine was excreted as unchanged compound, with traces of 13-OH-lupanine. Most subjects exposed to 13α-OH-lupanine excreted the unchanged compound, whereas two subjects excreted 14% and 34% as lupanine. Because no difference between CYP2D6 PM and EM individuals was observed, the CONTAM Panel concluded that, unlike in the case of sparteine, there is no clear indication for an involvement of CYP2D6 in the metabolism of lupanine or 13α-OH-lupanine.

There is no information on whether conjugated metabolites might be formed from lupanine and its metabolites.

3.1.1.2. Farm animals, horses and companion animals

There is little information in farm animals, horses and companion animals on the absorption, distribution, metabolism, and excretion (ADME) of the QAs considered in this Scientific Opinion. Most studies deal with wild lupins from non-European countries (i.e. *L. caudatus*, *L. leucophyllus* and *L. argenteus*) containing teratogenic alkaloids (e.g. anagyrine) which are of no interest for the current study.

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21 The carbon atom at which the hydroxylation took place is unspecified in the paper. However, the CONTAM Panel assumed that this is the 13-OH-lupanine.
opinion. Such studies will be taken into consideration in the following section only in the case they provide information on the fate of QAs considered in this Scientific Opinion.

Cattle

In an in vitro study aimed at investigating the impact of rumen microbiota on L. luteus alkaloids, Aguiar et al. (1998) incubated lupanine or sparteine, both at 1 mM concentrations, with cattle ruminal liquor. No significant metabolic degradation of either alkaloid was observed up to 36 h incubation.

Blood plasma levels of alkaloids from L. caudatus were studied in single dosed cattle, sheep, and goats of unspecified breed and age (Gardner and Panter, 1993). Aerial plant parts were dried and ground and QAs were measured with a GC-FID method. The plant material had a total alkaloid concentration of 19,200 mg/kg, and consisted of 8% lupanine, 22% 5,6-dehydrolupanine, 22% anagyrine and three unidentified alkaloids. Blood samples were collected up to 56 h post-dosing. As regards cattle, cows (N = 2) received 3 g dried plant material/kg bw. Although the alkaloids could be detected in plasma as soon as 15 min after dosing, lupanine as well as 5,6-dehydrolupanine appeared to be characterised, contrary to humans and experimental animals, by a slow absorption and a protracted elimination; indeed both compounds peaked approximately 8 h after treatment and the levels remained ‘moderately high up to 28 h post-dosage’.

The plasma disposition of lupin alkaloids was examined in cows (unspecified age and breed) with (group A, N = 6) or without (group B, N = 6) a history of delivery of calves affected by lupin-induced arthrogryposis (Gay et al., 2004). Dried and ground L. leucophyllus (aerial plant parts) was used with a measured (GC-FID) total alkaloid concentration (on a dried plant basis) of 16,700 ± 1,700 mg/kg. The composition consisted of six alkaloids, of which two remained unidentifed, 5,6-dehydrolupanine (3,900 ± 400 mg/kg), lupanine (1,000 ± 100 mg/kg), 11,12-seco-12,13-didehydromultiplorine (2,500 ± 200 mg/kg) and anagyrine (3,300 ± 300 mg/kg). Animals receiving a single dose of 2 g plant material per kg bw by gavage, were subjected to blood sampling up to 48 h post-dosing and the main toxicokinetic (TK) parameters were measured for the alkaloids mentioned above, except for 11,12-seco-12,13-didehydromultiplorine that was present only at very low concentrations. No differences between the two groups were observed and similar TK values were found for 5,6-dehydrolupanine and lupanine. The data point to a slow absorption, with the time to reach the maximum plasma concentration (T_max) ranging between 10 and 24 h, and a rather long persistence as indicated by a calculated mean residence time (MRT) in the range 29–149 h.

In a more recent study aimed at identifying reliable biomarkers of exposure to teratogenic lupins (Green et al., 2015a), Holstein steers (N = 4, 289 ± 13 kg bw) were orally dosed once with dried ground L. leucophyllus (aerial plant parts). The material was analysed by GC–MS and had a total alkaloid concentration of 18,660 ± 1,130 mg/kg. Four alkaloids were present: lupanine (6.90 ± 0.40 mg/kg), 5,6-dehydrolupanine (3.94 ± 0.16 mg/kg), anagyrine (5.14 ± 0.39 mg/kg) and an unidentified alkaloid (based on the molecular weight (248 g/mol) it can be assumed to be a QA). The calculated dosages were 17.2, 9.9, 12.9, and 6.7 mg/kg bw, respectively. Blood samples were taken up to 96 h after dosing. Lupanine was slowly but extensively absorbed reaching the highest maximum plasma concentration (C_max) (5.056 ± 1,094 ng/mL) and the slowest T_max (15.5 ± 7 h) among the four examined compounds, also showing a relatively long elimination half-life (7 ± 0.5 h). By contrast, 5,6-dehydrolupanine seemed to be absorbed to a much lower extent (632 ± 141 ng/mL) but in a more rapid manner (T_max 3.5 ± 0.5 h), but being also slowly excreted (6.7 ± 0.5 h).

A study was designed to disclose breed-related differences in lupin-mediated inhibition of fetal activity (expressed as number of movements/5 min) (Green et al., 2015b). To test the possible influence of lupin alkaloid disposition on the mentioned endpoint, the main TK parameters of lupanine, 5,6-dehydrolupanine and anagyrine were determined in cows. To this end, Angus (N = 6, 382 ± 39 kg bw) or Holstein (N = 5, 435 ± 46 kg bw) pregnant heifers at gestation day 49–51 were dosed once with 1.1 kg dried and ground L. leucophyllus (aerial plant parts) per kg bw. The plant material had the same alkaloid concentration as described above (Green et al., 2015a); blood sampling was carried out up to 72 h. While similar kinetic parameters were recorded for 5,6-dehydrolupanine, clear breed-related differences were observed for lupanine. T_max was higher in Holstein (12 h) than in Angus cows (8 h). In addition, at 8, 12 and 24 h, Holstein heifers had to up about 50% higher serum lupanine concentrations than their Angus counterparts. By contrast, as reflected by a longer elimination half-life (11.4 ± 1.4 h vs. 5.6 ± 0.5 h) lupanine tended to persist for a longer time in Angus than in Holstein heifers.
Sheep

The *in vitro* ruminal degradation of pure sparteine (up to 5.4 mM) or of sparteine extracted from ‘sweet’ or ‘bitter’ lupins was investigated (Lanca et al., 1994). In either case no significant metabolic degradation of sparteine was detected up to 24 h incubation.

In the study by Gardner and Panter (1993) cited above, sheep (N = 2) were administered with a single oral dose of a *L. caudatus* dried aerial plant parts (7.8 g/kg bw) (see above for further details). In blood, lupanine and 5,6-dehydrolupanine peaked at about 8 and 24 h, respectively; after treatment showing sustained high values of total alkaloids (range 2–3.5 $\mu$g/mL); blood levels of lupanine were about half of those of 5,6-dehydrolupanine. The concentrations of both alkaloids decreased slowly and both compounds could still be detected 56 h post-dosing.

In a further study (Lopez-Ortiz et al., 2004), ground silvery lupin (*L. argenteus*) (aerial plant parts) was analysed for its alkaloid concentration by GC-FID. It contained a total alkaloid content of 16,400 mg/kg. Besides three unidentified alkaloids, lupanine (8.2%), 5,6-dehydrolupanine (11.5%) and anagyrine (28.8%) were the main components. Ten Columbia ewes (age not reported) were administered with a single oral dose of ground plant (8.5 g/kg bw corresponding to 139 mg total alkaloids/kg bw) and blood samples were taken up to 60 h. Measurable amounts of alkaloids were already present in serum after 0.5 h and could be detected up to 48 h post administration. Peak levels were reached within three hours. Longer absorption- and elimination half-life values were recorded for 5,6-dehydrolupanine (1.67 ± 0.20 h and 7.88 ± 0.78, respectively) compared to lupanine (0.76 ± 0.20 h and 4.47 ± 0.55, respectively). Interestingly, longer elimination half-life values for lupanine were observed in sheep intentionally fed with a nutrient deprived diet resulting in a low body condition and treated as reported above.

Goats

The above cited paper of Gardner and Panter (1993) was the only report involving goats that could be identified. In that study, the toxicokinetics of QAs was compared to that of cows and sheep. Goats (N = 2) were treated with a single oral dosage of *L. caudatus* dried aerial plant parts (7.8 g/kg bw) (see above for further details). Plasma levels of lupanine and 5,6-dehydrolupanine peaked at around 3 h and declined slowly, being still detectable after about 30 h. While peak levels of either compound were comparable to those of cows, their concentrations were about half those displayed by sheep.

Pigs

In the study by Wasilewko et al. (1997), five barrows (i.e. male castrated pigs) of unspecified breed and an initial weight of 15 kg were equipped with a post-valvular T-shape caecum cannula and fed a standard diet free of QAs for 7 days. Pigs were subsequently exposed to a standard diet fortified with increasing lupanine concentrations (100, 150, and 200 mg/kg diet, each on three consecutive days) for 9 days. Samples of digesta (caecum content) and urine were collected (collection period not reported), pooled and analysed for alkaloid concentration using capillary GC and GC–MS (sensitivity not reported). No intact lupanine was found in the caecal content, suggesting an extensive absorption of the alkaloid; urine was found to contain mostly the parent compound along with the metabolites isolupanine and OH-lupanine (unspecified).23 No lupanine was detected in urine collected after 8 days on an alkaloid-free diet.

Rabbits

Liver microsomes were incubated with sparteine concentrations in the range 5 $\mu$M to 4 mM; the parent compound and its metabolites were determined by GC-FID. Only a single metabolite, 2,3-dehydrosparteine, was formed, the reaction showing a Michaelis constant (Km) of 36 ± 2 $\mu$M and a maximum velocity ($V_{max}$) of 70 ± 10 nmol/mg protein/30 min. The rate of metabolite formation was unaltered when microsomes from phenobarbital (PB)- or TCDD-induced rabbits were used and no metabolism occurred in the presence of purified flavin monooxygenase (FMO) (Ohnhaus et al., 1985). Taken together, results indicate that in the rabbit the formation of 2,3-dehydrosparteine involves a non PB- or TCDD-inducible CYP (possibly CYP2D). No further reports on QA toxicokinetics in rabbits could be retrieved.

23 Quantitative data not shown in the paper.
No adequate data on the toxicokinetics of QA could be retrieved for poultry, fish, horses, cats, and dogs.

3.1.1.3. Transfer

In general, due to their weak basic nature, plant alkaloids, including QAs, are expected to be excreted in dairy milk. Although indirect evidence has been provided of the excretion of teratogenic QAs both in cow (Panter and James, 1990) and goat milk (reviewed in Molyneux and Panter, 2009), no information could be retrieved about the mammary excretion of lupanine, 5,6-dehydrolupanine or other non-teratogenic QAs. It is not known whether feeding of lactating farm animals with lupins from *L. albus* or *L. angustifolius* results in occurrence of lupin alkaloids or their metabolites in cow’s, sheep’s or goat’s milk.

In the study by Vogt et al. (1987; see Section 3.1.4), there was no measurable transfer of alkaloids into meat or eggs of laying hens fed for 168 days a diet containing 16% of three different debittered lupin grists, namely *L. albus*²⁴ P., *L. albus*²⁴ G. or *L. mutabilis*, with a QA concentration ranging from 8 to 60 mg total alkaloids/kg grists, corresponding to 1.1–9.6 mg total alkaloids/kg diet. However, the Panel noted that the animal QA exposure via feed in this study is low and does not allow to assess possible transfer rates.

3.1.1.4. Main biotransformation pathways of quinolizidine alkaloids

Figure 4 summarises information on the biotransformation of sparteine and lupanine (mainly oxidative) in humans, pigs, rats and rabbits. There is no information on whether conjugated metabolites might be formed from sparteine and lupanine and their metabolites. For other QAs, a general picture of their biotransformation is lacking.

![Biotransformation of sparteine and lupanine in human, rat, pig and rabbit](image)

**Figure 4:** Biotransformation of sparteine and lupanine in human, rat, pig and rabbit

3.1.1.5. Summary remarks on toxicokinetics

**Humans and experimental animals**

There is scant information on QA toxicokinetics in rats and humans except for sparteine and lupanine. In humans and rats, sparteine and lupanine are rapidly absorbed, widely distributed and rapidly eliminated in urine mainly unchanged and as oxidised metabolites. Sparteine undergoes a CYP2D-mediated oxidation to 2,3-dehydrosparteine in rats, which are also able to oxidise sparteine into lupanine. In humans, the CYP2D6-mediated production of both 2,3-dehydrosparteine (major) and

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²⁴ Referred to as *L. alba* by the authors.
5,6-dehydrosparteine (minor) is reported. About 5–10% of Caucasians are PMs, as they are defective in CYP2D6 activity due to genetic polymorphisms, and these individuals eliminate sparteine more slowly. Lupanine is partly biotransformed to a hydroxyl derivative, presumably 13-OH-lupanine in rats through an unknown pathway. Lupanine is mainly excreted unchanged in humans with some formation of 13-OH-lupanine with no apparent involvement of CYP2D6. The de-hydroxylation of 13-OH-lupanine to lupanine is reported to occur in some individuals. There is no evidence of the formation of conjugated metabolites of sparteine, lupanine or their metabolites in rats and humans.

**Farm animals, horses and companion animals**

Little is known on QA ADME in farm animals, horses and companion animals; most of the published studies are concerned with teratogenic lupins also containing lupanine and 5,6-dehydrolupanine and just one *in vivo* study has been performed with the pure compounds (i.e. lupanine in pigs). Very limited information is available for other QAs, including sparteine.

No metabolic degradation of sparteine was found to occur upon the incubation with bovine and ovine ruminal fluid. By contrast, sparteine undergoes a CYP-mediated oxidation to 2,3-dehydrosparteine in rabbit liver microsomes.

As regards ruminants, in the majority of the reported studies blood levels recorded in cattle after a single exposure to different lupin species indicate, contrary to humans and experimental animals, a slow but extensive absorption and a slow elimination of lupanine and 5,6-dehydrolupanine, with possible breed-related differences. Under similar exposure conditions, a comparable kinetic behaviour was observed for both alkaloids in sheep and goats, the former exhibiting comparably higher and more sustained levels than cows and goats. In pigs, lupanine seems to be extensively absorbed and excreted in urine mainly unmodified together with the metabolites isolupanine and OH-lupanine. There is no evidence of the formation of conjugated QA metabolites in ruminants, pigs and rabbits.

No adequate data on the toxicokinetics of QAs for poultry, fish, horses, cats and dogs were identified.

Possible transfer to milk should be considered and no information is available to assess possible transfer rates of QAs in food of animal origin.

### 3.1.2. Toxicity in experimental animals

For studies reporting only concentrations of the toxin in the feed, doses have been calculated to mg or µg/kg bw per day following the respective EFSA guidance (EFSA FEEDAP Panel, 2012).

#### 3.1.2.1. Acute toxicity

An overview of the acute toxicity studies with QAs is presented in Appendix H.1.

*D*-lupanine, isolated from *L. angustifolius* seeds and purified by recrystallisation (identification by refractive index; final purity not reported), was injected i.p. in mice, rats and guinea pigs at concentrations of 15 and 100 mg/mL. Minimal lethal dose (MLD) values were 75–85, 180–192 and 210–225 mg/kg bw in mice, rats and guinea pigs, respectively. The dose killing 50% of the animals (LD<sub>50</sub>) for mice was 80 mg/kg bw. Fatally dosed animals suffered from paralysis, with intermittent convulsive movements, then complete exhaustion. Necropsy revealed that death resulted from asphyxiation (no apparent liver damage or other pathological lesions were noticed) and all animals seemed to die from respiratory failure (Gordon and Henderson, 1951).

Lupanine isolated from *L. albus* seeds and purified by column chromatography and recrystallisation (identification by 2D-TLC and IR; final purity not reported) and commercial sparteine were administered orally or i.p. to male Swiss mice. Oral LD<sub>50</sub>s were 410 and 220 mg/kg bw for lupanine and sparteine, respectively, while i.p. administration led to LD<sub>50</sub>s of 175 and 36 mg/kg bw, respectively. Identical symptoms (trembling, tonic-clonic seizures) were observed for both alkaloids and death was caused by breathing arrest. The lower toxicity of lupanine vs sparteine was confirmed in guinea pigs (intravenous (i.v.) administration), in which a MLD of 78 and 27 mg/kg bw was identified for lupanine and sparteine, respectively. In view of the similarity of the pharmacological properties of sparteine and lupanine, the authors conclude that the lower toxicity of lupanine is probably due to differences in bioavailability and/or pharmacokinetics (Yovo et al., 1984).

Oral LD<sub>50</sub> of alkaloid extracts from *L. angustifolius* (cv. Fest) seeds (alkaloid profile: 49% lupanine, 39% 13α-OH-lupanine, 10% angustifoline, 0.7% α-isolupanine) was 2,279 mg/kg bw for fed rats and 2,401 mg/kg bw for rats fasted for 16–18 h before dosing. Lupanine and 13α-OH-lupanine were further purified from the alkaloid extracts by recrystallisation and column chromatography (purity by
GC: > 99.5%). For lupanine, the LD$_{50}$ by oral administration to fasted male Wistar rats was 1,664 mg/kg bw. Administration of lupanine and 13α-OH-lupanine i.p. to rats identified LD$_{50}$s of 177 mg/kg and 199 mg/kg, respectively. After administration of a lethal oral dose of alkaloids, rats displayed nervous signs within 2–16 min and died within 2–23 min. Signs included tremor, followed by convulsions, cyanosis, collapse and death irrespective of the route of administration. Post-mortem analyses identified congestion of the liver and lungs in some animals. Surviving rats from all treatments showed no signs of clinical toxicity (Pettersson et al., 1987).

Stobiecki et al. (1993) extracted seeds of *L. angustifolius* and *L. albus* and these extracts (containing 10% alkaloids in the dry extract) were administered by gavage to male and female mice (inbred strain 129/AoBoy/Iiw, unspecified number of animals). No deaths were recorded and an LD$_{50}$ of > 4,000 mg lupin extract/kg bw (corresponding to > 400 mg alkaloids/kg bw) was established. The CONTAM Panel noted that it is not clear from the study how alkaloids were measured in these extracts.

A comparative study was conducted of the effects of extracts from *L. mutabilis* Sweet seeds (alkaloid profile: 20% sparteine, 59% lupanine and 21% 13α-OH-lupanine), lupanine isolated from *L. mutabilis* seeds and purified by column chromatography (purity checked by TLC and GC–MS) and sparteine sulfate pentahydrate. Five doses (50, 100, 125, 250 or 500 mg/kg) were i.p. injected in male Swiss mice and acute toxicity and maximum non-lethal dose (LD$_{0}$) were determined 8 days later (Pothier et al., 1998). LD$_{50}$s for i.p. administration were 25 mg/kg for sparteine and 64 mg/kg for lupanine and lupin extracts. Lethal i.p. doses (LD$_{100}$) were 100 mg/kg for sparteine and 250 mg/kg for lupanine and lupin extracts. The clinical signs of poisoning (shaking, excitation and convulsions) were the same for the three compounds.

**Summary remarks on acute toxicity**

Mice appear to be more sensitive than rats to acute toxicity (by i.p. and oral administration) of lupanine. A comparison of the acute toxicity of lupanine and 13α-OH-lupanine in rats (by i.p. administration) showed similar LD$_{50}$ values. In contrast, when comparing results from the same route of administration, several reports on mice and guinea pigs indicate that sparteine is approximately two- to three-fold more acutely toxic than lupanine, regardless of the way the acute toxicity is expressed. Finally, acute symptoms following oral administration of these QAs are similar in rats, mice and guinea pigs, with death resulting from respiratory failure and toxic effects affecting the central nervous system (CNS).

**3.1.2.2. Repeated dose toxicity studies**

The CONTAM Panel identified five repeated dose toxicity studies in which rats received a lupin-based diet. No studies in other experimental animals were identified. These studies are described below in detail and are summarised in Appendix H.2. No repeated dose toxicity studies on individual QAs were found. In addition to the papers described below, several other papers were identified which were considered of low relevance for the risk assessment due to a lack of information on the alkaloid concentration in the feed, poor reporting or unsuitable experimental design which focused on studying nutritional benefits of lupin seeds or mechanistic insights in experimental models of human disease. A description of these studies is provided in Appendix H.3.

In a 112-day feeding study, three groups of Charles River rats (21–23 day old) were given a diet containing casein (control group), ground seeds (58%) of *L. albus* (510 mg lupin alkaloids/kg, equivalent to 45 mg/kg bw per day) and ground seeds (51%) of *L. luteus* (910 mg lupin alkaloids/kg, equivalent to 81 mg/kg bw per day, no specification of QAs; total lupin alkaloid content measured by titration) (Ballester et al., 1980). Food and water were available *ad libitum* and dietary intake and body weights were recorded weekly. Animals fed either lupin seeds at 10% protein diets without DL-methionine performed poorly (low growth and protein efficiency ratio). In contrast when 20% protein diets supplemented with 0.3% DL-methionine were compared *L. luteus*-fed animals gained weight at rates similar to those fed with casein and a slight, but not statistically significant decrease was observed for *L. albus*-fed animals. At the end of the 112-day feeding period, no differences among the three groups were found in organ-to-body weight ratios of liver, kidneys, spleen, heart and adrenals. Histopathological examinations of liver, kidneys and lungs did not reveal any significant differences with the control group. The CONTAM Panel noted that no effects were observed at the only tested dose levels of 45 and 81 mg lupin alkaloids/kg bw per day, for *L. albus* and *L. luteus*, respectively.

A diet based on flour (33.6%) prepared from water-debittered grains of *L. mutabilis* and containing trace amounts of lupin alkaloids (160 mg/kg, equivalent to 14.4 mg/kg bw per day as determined by
Quinolizidine alkaloids in feed and food

GC-NPD, no specification of QAs was tested in Sprague-Dawley rats (21 days old) for 12 weeks (Schoeneberger et al., 1987). The protein content was 15% and the diet was supplemented with 0.2% DL-methionine. The control group was fed a 15% protein casein diet. Rats fed on lupin diet did not show any statistically different growth performances (body weight, food consumption) in comparison to the control group. No consistent indications of significant physiological differences (haematological and biochemical parameters) were recorded. Similarly, absolute and relative weights and histopathological examinations of some organs (liver, kidney, heart, spleen and brain) were unaffected by the alkaloids. The CONTAM Panel noted that no effects were observed at the only tested dose level of 14.4 mg lupin alkaloids/kg bw per day for L. mutabilis.

Diets based on L. angustifolius (cv. Fest) flour (554 g/kg diet) which had been spiked with extracts from L. angustifolius seeds to provide dietary concentrations of 250, 1,050 and 5,050 mg lupin alkaloids/kg diet (equivalent to 25, 105 and 505 mg/kg bw per day) was fed to three groups of Sprague-Dawley rats (3 weeks old) for 90–98 days (Butler et al., 1996). No control group was included but a reference group receiving the same lupin flour-based diet containing background levels of alkaloids (50 mg lupin alkaloid/kg diet, corresponding to 4.5 mg/kg bw per day) derived from the background level of alkaloids in lupin flour. The alkaloid profile of the flour was lupanine (44.2–44.6%), 13α-OH-lupanine (41.9–42.4%), angustifoline (9.9–10.9%) and α-isolupanine (1.0%) (analytical method not reported). The diets were supplemented with 0.6% methionine. No mortality was recorded during the study. A slight transient reduction in body weight was recorded in the male group treated with 250 mg/kg diet. Food intakes were generally equivalent to those of the reference group. Haematological investigations gave little evidence of consistent effects (changes were small, often of transient nature and characterised by lack of dose response). Clinical chemistry measurements revealed no differences between lupin-fed rats and the reference group. Relative liver weights were increased in female rats at all doses (4% increase and no dose response). Altered foci of liver parenchymal cells were seen in 1/20 females and 1/20 males in the lowest dose group, 1/20 males in the mid-dose group, 5/20 females in the highest dose group and none in the reference group. Cells comprising these foci were basophilic or large pale-staining cells, characterised by nuclei with prominent nucleoli and occasional mitotic figures (usually called altered hepatic foci). However, the Panel noted that these effects were not observed in a follow-up study described below.

Diets mixed with extracts from L. angustifolius (cv. Fest) containing lupin alkaloids at dose levels of 0, 100, 330, 1,000 or 5,000 mg/kg (equivalent to 0, 10, 33, 100 and 500 mg/kg bw per day) were fed to groups of Sprague-Dawley rats (4 weeks old) for 90 days (Robbins et al., 1996). The alkaloid profile consisted of lupanine (49.5–50.7%), 13α-OH-lupanine (38.4–39.2%), angustifoline (8.8–10.7%) and α-isolupanine (1.3–1.4%) (analytical method not reported). The diets were supplemented with 0.3% methionine. The control basal diets were commercial preparations with and without 4.5% maltodextrin. A statistically significant decrease in body weights was observed in both male and female rats fed a diet containing 500 mg/kg bw per day lupin alkaloids in comparison to the control diet groups. The body weight of both male and female rats fed 100 mg/kg bw per day were also lower, although the difference was statically significant only on a few time points. Feed intakes of both sexes given 100 or 500 mg/kg bw per day were consistently lower than the control groups. Some changes (with no dose response) were observed in haematological parameters and in clinical chemistry findings. The authors noted that these fell within the normal range according to age and sex of the animals. Relative liver weights at 500 mg/kg bw per day in both males and females were significantly higher (8–9%) than in control groups. Kidney weights showed some inconsistent and dose-unrelated differences between sexes. The histopathological examination of liver, heart and bone marrow was unremarkable and none of the altered foci of liver cells reported by Butler et al. (1996) were reproduced in this study. The CONTAM Panel noted that the reduced body weight gain already recorded at 100 mg/kg bw per day might be due to reduced palatability and feed intake. Based on the increased relative liver weight observed at 500 mg lupin alkaloids/kg bw per day, the CONTAM Panel identified 100 mg lupin alkaloids/kg bw per day as a dose of lupin alkaloids present in the studied L. angustifolius cultivar that is not associated with effects is rats.

Diets containing seeds of L. angustifolius were administered to male Wistar rats (3 weeks old, 81 g initial body weight, 8 animals/group) for 28 days. Diet materials were prepared from three L. angustifolius cultivars (25.8–27.1%), i.e. Baron, Zeus and Wersal, with different lupin alkaloid concentrations (360, 410 and 560 mg/kg, respectively) (Stanek et al., 2015). The composition of QAs (measured by GC-MS) was lupanine (62.4–70.2%), 13α-OH-lupanine (9.5–14.4%), angustifoline (8.7–10.6%), α-isolupanine (6.6–8.1%), tetrahydroxanthine (2.5–4.1%) and isoangustifoline (1.0–1.9%). The composition of the experimental diet was 10% protein and an alkaloid concentration...
of 90, 110 and 150 mg/kg feed (equivalent to 8.1, 9.9 and 13.5 mg/kg bw per day for Baron, Zeus and Wersal feed), respectively. The CONTAM Panel noted that previous experiments with 10% protein without \( \alpha \)-methionine supplementation showed that animals performed poorly (see Ballester et al., 1980). The control group received soybean meal. Diets containing the seeds of all \( L. \) angustifolius cultivars reduced the feed intake by 50% and the growth rate of experimental rats in comparison to the control group. Baron-, Zeus- and Wersal-based diets increased serum triacylglycerol (TG) levels, while decreased alanine transaminase (ALT) activity was observed only in Zeus- and Wersal-based ones. The liver weight of rats fed diets with \( L. \) angustifolius seeds decreased with increasing dietary alkaloid concentrations. Liver histopathology revealed parenchymatous degeneration of hepatocytes (more pronounced in rats fed Zeus and Wersal diets), and congestion of portal vessels and central veins (more severe in rats fed Baron and Zeus diets). The CONTAM Panel disregarded this study because of the nutritional imbalance and the large effects on feed intake and growth rate induced by relatively low levels of alkaloids from \( L. \) angustifolius.

**Summary remarks on repeated dose toxicity studies**

All the repeated dose studies with laboratory animals on QAs were carried out in rats. The CONTAM Panel identified two key 90- to 98-day studies performed in the same laboratory using \( L. \) angustifolius-based flour. The major QAs of the diet were lupanine and \( 13\alpha\)-OH-lupanine (about 40% each). Both studies reported an increase in relative liver weights. However, the induction of altered liver foci observed in the first study was not confirmed in the second one. Conflicting results were also obtained in the two studies on weight gains. Based on increased relative liver weight a NOEL of 100 mg alkaloids/kg bw per day was proposed in the second study.

No significant toxicological effects were reported in sub-chronic studies on \( L. \) albus-, \( L. \) luteus- and \( L. \) mutabilis-based diets at doses of 45, 81 and 14.4 mg alkaloids/kg bw per day, respectively, which were the only doses tested. It should be noted that lupanine is the major alkaloid contained in \( L. \) albus and \( L. \) angustifolius, while the more toxic sparteine is a major alkaloid present in \( L. \) luteus and \( L. \) mutabilis (see Table 1).

The PANEL noted that the only available studies on QAs repeated dose toxicity are in rats, the less sensitive species among experimental animals (see acute toxicity section). In addition, it is difficult to compare these studies because of differences in the sources of QAs (seed extracts vs flour), cultivars, presence of alkaloids other than QAs relevant for this opinion, and the control diets (commercial, lupin-based) used for comparison. Because of these reasons and some conflicting results reported in different publications from the same laboratory, the CONTAM Panel considered that these studies have a limited value for the evaluation of QA toxicity.

**3.1.2.3. Neurotoxicity**

In an acute toxicity study in mice by Pothier et al. (1998), the effects of i.p. administration of lupanine and sparteine (64 and 25 mg/kg, respectively, representing the maximum non-lethal i.p. doses) on behaviour (i.e. psychomotor activity and exploration test), were investigated. Both alkaloids elicited a weak decrease in spontaneous activity and reduced locomotor activity indicating a slight sedative action on the CNS. Also, the interaction with drugs acting on the CNS and analgesic effects were studied. No effects were seen on the interactions with drugs such as stimulating (amphetamine, pentetrazol) or depressant (pentobarbital, chlorpromazine) drugs. Similarly, these alkaloids did not show any analgesic activity (on abdominal constrictions induced by acetic acid).

The effects of the intracerebroventricular (ICV) administration of QAs extracted from \( L. \) exaltatus and \( L. \) montanus seeds were studied in adult Swiss-Wistar rats (10 animals/group) receiving a daily ICV injection (10 \( \mu \)L) of sesame oil (control group) or QA extract on five consecutive days (Banuelos Pineda et al., 2005). Principal QAs present in \( L. \) montanus extracts were sparteine (35,000 mg/kg), lupanine (17,700 mg/kg), \( 3\beta\)-OH-lupanine (3,800 mg/kg) and \( 13\alpha\)-OH-lupanine (3,200 mg/kg). Extracts from \( L. \) exaltatus contained lupanine (5,800 mg/kg), \( 3\beta\)-OH-lupanine (1,500 mg/kg) and only a small amount of sparteine (284 mg/kg). Clinical symptoms immediately after QA administration included tachycardia, tachypnoea, piloerection, tail erection, muscular contractions, loss of equilibrium, excitation, and unsteady walk. The contralateral unlesioned left hemisphere of the brain was analysed at the end of the treatment. A high number of neurons with degenerative alterations (30–40% vs ~ 10% in the control group) were observed in the brains of QA-treated animals, thalamus, hypothalamus, and hippocampus being the most susceptible brain areas. These structures contain cholinergic neurons, structures related to cholinergic paths and structures of the basal ganglion that possess cholinergic receptors (muscarinic or nicotinic type). These neurotoxic findings support the
mode of action of QAs in the CNS proposed by Kinghorn and Balandrin (1984) and Schmeller et al. (1994). The authors conclude that neuronal damage induced by QAs from \textit{L. montanus} was more severe than that of \textit{L. exaltatus} (no quantification reported) in all the examined areas. These results are in agreement with the high content of sparteine present in QA extracts from \textit{L. montanus}.

Following sparteine administration either ICV or i.p. in Wistar rats (8 animals/group) for 5 days, Meraz Medina et al. (2017) found that brain structures of the CNS showing toxic effects were those mainly related to cholinergic pathways (frontal, frontoparietal and striate cortex, olfactory and amygdaloid area, ventromedial hypothalamic nucleus, cerebellar Purkinje cells and the Cornus Ammonis area 1 and 3 dentate gyrus regions of the hippocampus).

Sparteine-induced brain damage was investigated in neonatal Wistar rats (8 animals/group) receiving a single dose of 25 mg/kg bw by subcutaneous injections at postnatal day 1 and 3. Sparteine-induced damage to neurons in the cerebral motor cortex (analysed at 7, 14, 21 and 60 days) was visible at 7 days and persisted up to 21 days. Neuronal death was due to necrosis and was significantly reduced by pretreatment with atropine suggesting that cytotoxicity of sparteine is mediated by muscarinic acetylcholine receptors (mAChRs). Transient decreases in the expression of m1–m4 subunits mAChR both at RNA and protein levels were indeed demonstrated (Flores-Soto et al., 2006).

Yovo et al. (1984) investigated comparatively the toxicodynamics of sparteine and lupanine. Cats (n = 4) and dogs (n = 4) were injected i.v. with sparteine (cats: 0.5, 1.0, 1.5, 2.0 and 4.0 mg/kg bw; dogs: 5.0 and 7.5 mg/kg bw) and lupanine (cats: 1.0, 2.0, 3.0, 4.0 and 5.0 mg/kg bw; dogs: 5.0 and 7.5 mg/kg bw). Sparteine is more efficient than lupanine in antagonising secondary reflex hypertension caused by carotid occlusion and hypotension induced by electrical stimulation of pneumogastric nerve both in the cat and in the dog. Lupanine and sparteine show identical inhibitory action on nicotinic type hypertension induced by injection of acetylcholine (ACh) in atropine-treated dogs.

The CONTAM Panel noted that the method of application in the above described studies (in particular ICV administration) might be of limited relevance for assessment of the toxicity of QAs via the diet. However, they give an indication of the neurotoxic effects that QAs may cause and the related mode of action.

### 3.1.2.4. Genotoxicity

No binding or intercalation with DNA (as measured by DNA methylgreen release from DNA and inhibition of DNA polymerase I) was identified for several QAs (anagyrine, angustifoline, cytisine, 3β-OH-lupanine, 13α-OH-lupanine, lupanine, lupinine, 17-oxosparteine, sparteine, 13α-tigloyloxylupanine) (Wink et al., 1998).

At variance with alkaloids belonging to other chemical classes (e.g. indole and quinoline alkaloids) which bind/intercalate in DNA, cytisine, lupanine and sparteine were unable to induce apoptosis as identified by DNA fragmentation, in HL60 cells (Rosenkranz and Wink, 2007).

Lupanine did not induce gene mutations in \textit{Salmonella} Typhimurium strains TA97, TA98, TA100, TA102, TA1535, TA1538 in the presence or absence of exogenous metabolic activation (S9) (Santiago Quiles et al., 2010).

In the same study, negative results were also reported in these \textit{S. Typhimurium} strains with ethanol extracts of \textit{L. termis} seeds (the authors state that lupanine is the main alkaloid present in \textit{L. termis}). Ethanol extracts of \textit{L. termis} were also negative for mutation induction in L5178Y tk+/tk− mouse lymphoma cells and for micronuclei formation in the bone marrow of BalbC mice administered a single i.p. dose of \textit{L. termis} extracts (635 mg/kg bw corresponding to 50% of the LD50) (Santiago Quiles et al., 2010).

Sparteine ability to induce single-strand breaks (SSBs) was measured by Comet assays in a non-standard assay based on cultures of \textit{Tradescantia} staminal nuclei. A small increase in breaks was observed at a single dose. In the same study, ethanol extracts from \textit{L. mexicanus} and \textit{L. montanus} increased SSBs in the same cells at all tested concentrations, but with no dose dependency (Silva et al., 2014). The CONTAM Panel noted the limitations of the study, i.e. lack of dose response, no information on toxic effects and the use of a non-standard assay.

Unpublished data (BIBRA, 1986) showing negative mutagenic effects in \textit{S. Typhimurium} of alkaloid preparations from \textit{L. angustifolius} have been mentioned in reviews (Pettersson, 1998; Pilegaard and Gry, 2008) (the original report of BIBRA was however not available and thus the information could not be verified by the CONTAM Panel).
Summary remarks on genotoxicity

Very limited information is available on the genotoxicity of QAs, which is summarised in Appendix H.4. None of several QAs (anagyrine, angustifoline, cytisine, 3β-OH-lupanine, 13α-OH-lupanine, lupanine, lupinine, 17-oxosparteine, sparteine, 13α-tigloyloxylupanine) were able to bind or intercalate into DNA. Lupanine and extracts of L. termis (main alkaloid being lupanine) were unable to induce gene mutations in bacteria and in mammalian cells in vitro or micronuclei in vivo. The data on sparteine are too limited to conclude regarding genotoxicity.

3.1.2.5. Developmental and teratogenicity studies

Ballester et al. (1984) studied the effects of lupin feeding for 9 months in a multigeneration study in Wistar rats. The lupin diet containing 51.8 g flour from L. albus per 100 g diet (supplemented with 0.2% DL-methionine) was compared to a control diet containing the same protein levels (20%) obtained by defatted soybean flour, fishmeal and dried skimmed milk. The total alkaloid level of the test diet was 250 mg/kg (corresponding to 12.5 mg/kg bw per day). The results for the F0 generations have been reported in a previous study (Ballester et al., 1982; see Appendix H.3). F1 and F2 generations (descendants of the parent F0 generation mated at 12 weeks of age) were given the same diet as their parents. The growth rate of males fed the lupin diet both at the F1 and F2 generations was higher than that of the control group. No differences were observed in females. No significant changes in the relative weights of heart, spleen, kidneys, brain and gonads were observed with the exception of relative liver weights of both lupin-fed male and female rats being significantly lower than control animals. The histology of the liver, as well as that of the other organs, was, however, normal. There were no treatment-related haematological changes or changes in ALT and aspartate transaminase (AST) at any stage in either generation. Reproductive performance was also unaffected by a lupin-containing diet. The authors proposed that the difference in relative liver weights between lupin-fed male rats and control groups is due to the unusually high liver weights of the control group (not observed in previous studies from the same group using a casein diet as control group).

Kennelly et al. (1999) tested QAs extracted from rhizomes of blue cohosh (Caulophyllum thalictroides) in vitro rat embryo cultures. Following methanol extraction and purification of the extracts, QAs were analysed by GC–MS. The assay is an assessment of the overall growth and morphogenesis of organogenesis-staged rodent embryos. In this assay, rat embryos are dissected from the dams at gestation day 9.5 and cultured in medium together with test compounds for 45 h. α-Isolupanine and N-methylcytisine isolated from the extract were tested (purity not specified). α-Isolupanine was negative at all concentrations tested (0, 5, 20 and 80 μg/mL). In contrast, N-methylcytisine (tested at 5, 20, 50, 80 and 250 μg/mL) caused major malformations (open anterior neural tube, disturbed eye development and twisted tail) from 20 μg/mL onwards. Anagyrine (tested at 5, 20, 80 and 250 μg/mL) inhibited overall morphogenesis at the highest concentration tested but did not cause teratogenic effects (it should be noted however that skeletal development is not scored in this assay).

In conclusion, a two-generation study in rats indicate that a diet based on L. albus seeds did not affect the reproductive performance. In addition, an in vitro study in rat embryos assessing overall growth and teratogenic potential of purified α-isolupanine did not cause major malformations. No information is available on the effects on development and the teratogenic potential of other QAs.

3.1.2.6. Carcinogenicity

The CONTAM Panel did not identify carcinogenicity studies.

3.1.3. Observations in humans

The data presented here reflect the existing knowledge on effects observed in humans after exposure to lupin alkaloids or lupin seeds which results from intake for medicinal purposes, human studies in healthy volunteers or from case reports on accidental intake leading to intoxication.

The Panel noted that QAs, including sparteine and lupanine, are also known as components of the aerial parts of Cytisus scoparius (L.) Link (synonym: Sarothamnus scoparius (L.) W.D.J. Koch; vernacular name: Scotch broom; family: Fabaceae), for which medicinal uses have been reported (e.g. Thies, 1986). However, data obtained for C. scoparius are not considered relevant for this assessment due to differences in the pattern of QAs occurring in parts of C. scoparius compared to that of lupin seeds.
3.1.3.1. Pharmacodynamics

Among the QAs, sparteine is the only one for which human data from previous medicinal uses as an antiarrhythmic or oxytocic drug are available (Thies, 1986). However, existing data are limited.

**Sparteine**

**Knowledge from use as an antiarrhythmic agent**

Sparteine has been shown to diminish irritability and conductivity of the heart muscle as a sodium channel blocker in a comparable manner to quinidine and has been used therapeutically since 1873 for the treatment of cardiac arrhythmias (Thies, 1986; Blaschek et al., 2006). Thies (1986) describes that single doses of 15 to 20 mg sparteine (equivalent to 0.21 to 0.29 mg sparteine/kg bw for a person with a body weight of 70 kg) were introduced in the 1920s in the treatment of cardiac arrhythmias in France. However, Thies (1986) describes this dosage as below the threshold of pharmacological activity. According to Blaschek et al. (2006), antiarrhythmic properties have been reported for sparteine sulfate in an oral dose of as low as 20 mg (equivalent to 0.29 mg sparteine sulfate/kg bw for a person with a body weight of 70 kg) without clinical studies being available. In later years, (-)-sparteine sulfate was used in the treatment of tachycardia in single doses of 100 mg/tablet or 200 mg/tablet as a Class I antiarrhythmic (Rote Liste, 1983; Thies, 1986; Blaschek et al., 2006). The maximum single therapeutic dose was indicated to be at 4 mg/kg bw (Thies, 1986; Blaschek et al., 2006). According to clinical experiences, a single dose of 100 mg (equivalent to 1.4 mg/kg bw for a person with a body weight of 70 kg) still could not ensure a reliable antiarrhythmic effect. However, this could be achieved with a single oral dose of 150–200 mg (equivalent to 2.1–2.9 mg/kg bw for a person with a body weight of 70 kg). The recommended daily dosage for (-)-sparteine sulfate ranged from 800 to 1,000 mg per day for short-term use and from 400 to 500 mg per day for long-term therapy, the daily doses always being divided into 4–5 single doses (Rote Liste, 1983). Individual variations in sensitivity to sparteine sulfate are explained by the genetic polymorphism, which the isoenzyme CYP2D6 underlies. This isoenzyme is responsible for the metabolic oxidation of sparteine to 2,3-dehydro- and 5,6-dehydrosparteine (Eichelbaum et al., 1986; Thies, 1986; see also Section 3.1.1.1). Due to the existing genetic polymorphism, these two metabolites cannot be formed by 5% of the population who thus 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. These PMs were considered as a group at higher risk to develop adverse effects, which include gastrointestinal disturbances, central nervous disorders, dizziness, head pressure, accommodation disturbances in the eye, bradycardia, negative inotropy, hypertonia, changes in haemogram and liver enzymes. Use during pregnancy is considered as contraindication (Rote Liste, 1983; Thies, 1986; Blaschek et al., 2006; Aktories et al., 2009).

From the interindividual differences in sensitivity between PMs and EMs, the Panel concludes that the pharmacological activity is expected to be associated with the parent compound (-)-sparteine and lost upon biotransformation.

**Knowledge from use as an oxytocic agent**

Sparteine exhibits oxytocic properties, leading to a muscular stimulation in the virginal, puerperal and gravid uterus. During the expulsive phase of birth, sparteine induces rhythmic contractions of the uterus (Thies, 1986).

Dipont investigated the effect of orally administered sparteine sulfate on uterine contractility and if the treatment reduces the duration of labour during childbirth (Dipont, 1971). Sparteine sulfate was given to 427 women in the first stage of labour in the form of a 20% solution in drops in single doses of 100–150 mg by repeating dosage every hour if needed, up to a total dose of 600 mg. Compared to the control group, which comprised 98 labouring women, a statistically significant shortening of the duration of the first stage of labour was achieved by the treatment. The shortening amounted on average to 2 h and 8 min and was due to pronounced oxytocic activity. The average dose of sparteine

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25 According to Milne (2018) and The Merck Index (2006), it is assumed that sparteine sulfate is used in the form of the pentahydrate (MW: 422.54). A single dose of 20 mg sparteine sulfate pentahydrate (equivalent to 0.29 mg/kg bw for a person with a bw of 70 kg) would then correspond to a single dose of 11.09 mg sparteine (MW: 234.38) (equivalent to 0.16 mg/kg bw for a person with a bw of 70 kg).

26 A single dose of 100 mg sparteine sulfate pentahydrate (equivalent to 1.4 mg/kg bw for a person with a bw of 70 kg) would then correspond to a single dose of 55.47 mg sparteine (MW: 234.38) (equivalent to 0.78 mg/kg bw for a person with a bw of 70 kg).
sulfate administered was 402.5 mg. In this study the use of sparteine sulfate did lead neither to an increase of operative deliveries, nor to an increase in the number of perinatal complications, nor did it have a negative effect on the fetus. An adverse effect reported for the pregnant women was vomiting, which was associated by the author with the bitter taste of the sparteine sulfate (Dipont, 1971).

Sparteine sulfate was administered by intramuscular injection in repeated doses of 100–150 mg (equivalent to 1.4–2.1 mg/kg for a body weight of 70 kg) to intensify labour (Gessner and Orzechowski, 1974). However, due to the unpredictable occurrence of ‘tetanic’ uterine contractions, the use of sparteine sulfate as an oxytocic was banned by the FDA (1979).

**Intoxication**

The symptoms of intoxication with sparteine, for which PMs are at higher risks, are dizziness, drowsiness, headache, sweating, mydriasis and myasthenia. Via the paralysis of the phrenic nerve endings, sparteine produces in oral doses from 40 mg/kg bw onwards peripherally a curare-like effect which may lead to respiratory arrest and death. In cases of intoxication, where peripheral respiratory paralysis has been overcome by artificial ventilation, higher oral doses from 90 mg sparteine/kg bw onwards, induce bradycardia with the risks of fatal outcome due to cardiac arrest (Thies, 1986; Blaschek et al., 2006; Aktories et al., 2009).

A child of the age of 2 years and 4 months died 3 h after accidental ingestion of 413 mg of sparteine (according to the author equivalent to approximately 30 mg of sparteine/kg bw) in the form of a medicinal product. After 2 h, the child became conspicuously tired. Respiratory paralysis was observed followed by intense pulse acceleration. Tonic seizures occurred repeatedly before death set in (Schmidt, 1961).

A 4-year-old boy with a body weight of 15 kg was intoxicated after accidental ingestion of 100 mg sparteine sulphate/kg bw in the form of tablets. A 4-year-old boy with a body weight of 15 kg was intoxicated after accidental ingestion of 100 mg sparteine sulphate/kg bw in the form of tablets. Three hours later and after admission to the hospital, extrasystoles, a heart rate of 95 per min and gasping were observed, followed by vomiting and cardiorespiratory arrest. The boy survived after cardiopulmonary resuscitation was delivered (Landes and Robl, 1967).

3.1.3.2. Human studies

**Lupanine, 13α-OH-lupanine**

In a randomised kinetic study with a cross-over design described in detail in Section 3.1.1.1, oral doses of 10 mg of lupanine or 10 mg 13α-OH-lupanine (each equivalent to 0.143 mg/kg bw for a person with a body weight of 70 kg) were administered with a two-week wash-out phase to eleven volunteers. With respect to the expression of CYP2D6, seven of them were classed as EMs and four as PMs. No adverse effects were observed in any of the test subjects, blood pressure and heart rate remaining unaffected (Petterson et al., 1994).

3.1.3.3. Case reports on intoxication with lupin seeds

From the standard literature, it is known that symptoms of lupin seed intoxication set in 20 min after consumption with maximum effects being observed after 4–5 h. Typical symptoms are of neurological nature and may additionally manifest in the digestive and/or cardiovascular systems. Moderate cases of intoxication represent themselves by mydriasis, dizziness, nausea, dryness of the mouth, stomach aches, vomiting, diarrhoea and/or cardiac complaints. For severe cases of intoxication prominent tiredness accompanied by curare-like paralysis and convulsions 2–3 h after intake have been described. Death can occur by respiratory paralysis or cardiac arrest and also by choking (Schmidlin-Mészáros, 1973; Blaschek et al., 2006; Forrester, 2006).

In general, the alkaloid pattern of the lupin seeds, which were reported to induce the intoxication, is unknown and often the botanical origin of the seeds (e.g. lupin species and variety) is not indicated.

According to the available literature, including review articles and risk assessment monographs published by Food Authorities, the number of known cases of intoxication associated with the intake of lupin seed-based food is limited (e.g. Schmidlin-Mészáros, 1973; ANZFA, 2001; Pilegaard and Gry, 2008; BfR, 2017a).

27 Assuming the intake of sparteine sulfate in the form of the pentahydrate, 100 mg sparteine sulphate pentahydrate/kg bw would correspond to 55.47 mg sparteine/kg bw.
In the following, focus is put on case reports on poisonings with relevance for the risk assessment of the intake of QAs with lupin seed-based food, e.g. giving information on dose-effect relationships.

More than 10 published reports on poisoning cases in humans with seeds of the genus Lupinus or preparations thereof have been identified during the literature search (see Section 2.1). However, the CONTAM Panel is aware that this list may not be complete. The few cases which allow for estimation of alkaloid doses, to which subjects have been exposed, are summarised in Table 2. Other cases for which a quantification of alkaloid exposure was not possible (Smith, 1987; Lowen et al., 1995; Tsiodras et al., 1999; Di Grande et al., 2004; Sarikaya and Tettenborn, 2006; Litkey and Dailey, 2007; Kurzbaum et al., 2008; Pingault et al., 2009; Awada et al., 2011; Jamali, 2011; Daverio et al., 2014) are not dealt with here in detail. In some of the case reports, authors report on inadequate debittering of lupin seeds before consumption (Smith, 1987; Lowen et al., 1995; Litkey and Dailey, 2007; Kurzbaum et al., 2008; Awada et al., 2011; Jamali, 2011; Daverio et al., 2014). One case of intoxication was even associated with the drinking of 0.5 L water from debittering of lupin seeds (Marquez et al., 1991). Schmidlin-Mészáros (1973) and Schmidt (1961) summarise three cases from older literature of lethal intoxication of children after the intake of lupin seeds (see Table 2). Furthermore, three cases of poisoning with higher alkaloid dose estimates per kg bw are described in adults, who survived. This suggests that children may be more susceptible to intoxication with lupin alkaloids than adults and that doses from 10 mg/kg bw onwards have to be considered to be lethal in children (Schmidt, 1961; Schmidlin-Mészáros, 1973; Malmgren et al., 2016).

The available data do not allow drawing conclusions on the botanical origin of the lupin seeds involved in intoxication. However, Schmidlin-Mészáros (1973) and Awada et al. (2011) name *L. albus* or *L. luteus*, respectively, as species whose seeds have led to cases of poisoning.

**Table 2**: Summary of published reports on lupin seeds intoxication in humans which allow for quantitation of alkaloid intake

| Affected subject | Intake of seeds (Lupin species and alkaloid level of seeds) | Estimated dose of total alkaloids | Symptoms and outcome | Reference |
|------------------|-------------------------------------------------------------|----------------------------------|----------------------|-----------|
| 17-month-old toddler, bw: about 8 kg | 5–10 g; (seeds of unknown botanical origin contained more than 1% sparteine and lupinine) | 12–25 mg/kg bw | Paralysis, Death | Petraroia (1926) (as cited by Schmidlin-Mészáros, 1973) |
| 10-year-old boy, bw: about 30 kg | About 25–30 g; (*Lupinus albus*) | About 17–20 mg/kg bw | Gastric symptoms, mydriasis, clonic seizures, dyspnoea, cyanosis; Death after 3 h | Fischer (1940) (as cited by Schmidlin-Mészáros, 1973 and Schmidt, 1961) |
| 18-month-old toddler, bw: about 9 kg | 5–10 g | 11–22 mg/kg bw | No symptoms reported | Fischer (1940) (as cited by Schmidlin-Mészáros, 1973) |
| Adult (female), bw: about 55 kg | 70–80 g dry seeds (*Lupinus albus*) | 25–29 mg/kg bw | Strong gastric pain, vomiting, disturbed vision, strong mydriasis, dry throat, imperceptible pulse, agitation, dyspnoea | Abbozzo (1952) (as cited by Schmidlin-Mészáros, 1973) |
| Adult (male), bw: about 65 kg | 100–(150) g dry seeds (total alkaloid level: 2%; lupanine concentration of total alkaloids: 75–80%) | About 31–46 mg/kg bw | Nausea, mydriasis, coma; Recovery | Schmidlin-Mészáros (1973) |
Overall, it can be concluded that intoxication with lupin seeds do not occur frequently and rarely lead to a fatal outcome. In poisoning cases, the estimated total alkaloid intakes range from about 10–50 mg/kg bw. For children, lethal cases of poisoning have been described from 10 mg/kg bw onwards. In several publications, intoxication have been associated with inadequate debittering of lupin seeds by consumers (Smith, 1987; Lowen et al., 1995; Litkey and Dailey, 2007; Kurzbaum et al., 2008; Awada et al., 2011; Jamali, 2011; Daverio et al., 2014; Malmgren et al., 2016). No case of poisoning has been identified which was attributed to consumption of industrially produced lupin seed-based food. The botanical origin of the lupin seeds involved in intoxication remains unclear in most case reports. However, Schmidlin-Mészáros (1973) and Awada et al. (2011) indicate that seeds of *L. albus* and *L. luteus*, respectively, have led to cases of poisoning.

### 3.1.4. Adverse effects in farm animals, horses and companion animals

Little is known about the effects of QAs in farm animals, horses and companion animals. As mentioned above (see Section 3.1.1.2) wild lupins contain teratogenic alkaloids including QAs (e.g. anagyrine) which may be ingested upon grazing by ruminants. Most of the available literature deals with such effects (for a review, see Panter et al., 1999). However, since lupins used in European countries as feed do not contain teratogenic compounds, such effects will not be considered further.

The CONTAM Panel identified five papers studying the effect of single oral dosing of plant material from *L. caudatus* or *L. argenteus* in ruminants (see Appendix H.5). In these studies, the animals were exposed to a mixture of lupin alkaloids that consisted of less than 50% of QAs that are the subject of this opinion. These studies are therefore considered to be of limited relevance for the opinion. In addition, several other studies showing a poor experimental design were identified, which are also reported in Appendix H.5.

#### 3.1.4.1. Ruminants

**Cattle**

A study (Johnson et al., 1986) was conducted on 12 Jersey heifers (average weight 107 kg), which were randomly allocated to two groups receiving either a diet containing soybean (control) or a diet containing Tifwhite-78 lupin seeds (19.3%), a low alkaloid cultivar of *L. albus*, for 70 days. The seeds contained 680 mg lupin alkaloids/kg (dry matter (DM)) (measured by a GC method) and the resulting dietary concentration was 131 mg lupin alkaloids/kg feed (DM). Based on the reported dietary intake and weight, the CONTAM Panel calculated a corresponding dose of 4.5 mg/kg bw per day. Blood sampling was performed after 35 and 70 days of feeding. Feed consumption and body weight were measured daily. No statistically significant differences were observed in dietary intake, weight gain and feed efficiency. In addition, the lupin diet did not induce significant changes in serum biochemistry (Ca, P, Mg, total protein, albumin, BUN, total bilirubin, AP, AST, GGT, LDH). The CONTAM Panel concluded that heifers can tolerate lupin alkaloids present in the studied *L. albus* cultivar at a dietary concentration of 131 mg/kg, corresponding to a dose of 4.5 mg/kg bw per day. The CONTAM Panel noted that this was the only dose tested.
According to a randomised complete-block design, 25 Friesian dairy cows were offered one of the following diets with seeds from *L. albus* for 70 days: control, intact crushed lupin seeds (ICL) at 150 (ICL-15) or 300 (ICL-30) g/kg diet, and heat detoxified crushed lupin seeds (DCL) at 150 (DCL-15) or 300 (DCL-30) g/kg diet. Total alkaloids were extracted, but only the sum of the lupanine and 13α-OH-lupanine was measured (GC–MS); in ICL-15 and ICL-30 it was 3,800 and 8,000 mg/kg diet DM, respectively, while that of DCL-15 and DCL-30 was 2,100 and 5,200 mg/kg diet DM, respectively. Based on the reported dietary intake and assuming a body weight of 650 kg, the CONTAM Panel calculated corresponding doses of 72, 123, 52 and 111 mg/kg bw per day. Irrespective of the nature of the seeds (intact vs heat inactivated), cows exposed to the higher lupanine and 13α-OH-lupanine dosages (72, 111, and 123 mg QA/kg bw/day) had a lower feed intake (12.3, 13.9 and 10.0 kg/day), respectively. In addition, feed intake and milk yield were significantly depressed in cows on intact lupins as compared to those on detoxified lupins (Mukisira et al., 1995). The authors concluded that the reduction in feed intake could be attributed to lupanine and 13α-OH-lupanine. Based on this endpoint, it may be concluded that dairy cows tolerate a diet containing lupin seeds from *L. albus* at a concentration of 2,100 mg lupanine and 13α-OH-lupanine/kg, corresponding to a dose of 52 mg/kg bw per day.

**Sheep**

In the *in vitro* experiments cited above (Lanca et al., 1994), rumen microbial activity was not affected by the incubation for 24 h of either sparteine up to 5.4 mM or of finely ground *L. luteus* seeds (cv. Brava (bitter) or Topaz (sweet)) at three levels of incorporation (5, 10 or 15 g DM basis).

No relevant studies could be identified concerning the exposure to cultivated lupins.

**Goats**

No relevant studies could be identified concerning the exposure to cultivated lupins.

**3.1.4.2. Pigs**

Based on a review of the scientific literature, Cheeke and Kelly, 1989 concluded in that pigs are reported to be more susceptible than other species to QAs contained in SLs.

Case reports of lupin toxicosis have been described in pigs. Feed refusal, unthriftiness, lethargy, sporadic deaths, reduced litter size, stillbirth, increased postnatal death losses, poor performances and intestinal stasis were reported by three different pork producers feeding growing piglets or sows with dehulled hexane-extracted lupin seeds (most likely *L. albus* cv. Ultra, alkaloid content not reported) at dietary concentrations from 10% to 30% (Casper et al., 1991).

More recently (2016), a field case of lupin toxicosis has been described in northern Italy, affecting about 2,170 pigs of different categories (pregnant or lactating sows, gilts, fattening pigs) and involving several breeding farms. Poisoning was due to accidental contamination of grain legumes (peas) by bitter lupins (BLs) (*L. albus*). Total QA content of feed was determined by the University of Milan with a GC–MS method. There was a clear relationship between the QA concentration in the feed and the severity of clinical signs. A decrease in feed consumption was already noted after the exposure to dietary QA concentrations of 194 mg/kg; a more marked decline in feed intake was observed in animals offered diets containing 373 or 450 mg QA/kg. Some of the latter (fattening pigs, about 5%) also showed depression, recumbency, hypersalivation and vomiting along with a low mortality (about 1%) that was characterised at necropsy by torsion of the stomach and gastroenteric bloat. A remarkable reduction in feed intake, a higher incidence of clinically affected individuals (up to 50%) and a more severe clinical picture was instead reported in animals exposed to dietary QA levels of 866, 946 and 1,180 mg QA/kg feed, respectively. Mortality rose up to 20% in animals (lactating sows) fed with the highest QA levels (Boschin and Tesio, 2019, personal communication).

The tolerance of growing pigs to lupin alkaloids was tested in two different trials (Godfrey et al., 1985) in terms of changes in growth rate, feed intake, and feed conversion ratio. Total alkaloid concentration was measured by GC (method according to Priddles, 1983). In experiment 1, 9-week-old crossbred (Large White x Landrace) piglets (average weight 23 kg) were allocated to six groups receiving diets containing increasing amounts of lupin seeds (a mixture of *L. angustifolius* sweet cultivars) and consequently increasing total alkaloid concentrations (i.e. 120, 200, 280, 360, 440, 520 mg total alkaloids/kg feed, respectively) for 9 weeks. The corresponding doses calculated by the CONTAM Panel are 5, 8, 11, 14, 16 and 19 mg/kg bw per day, respectively. In experiment 2, piglets of...
the same breed (average weight 31 kg) were allocated to 3 groups receiving diets containing increasing amounts of BL seeds (L. angustifolius bitter cultivars) and consequently increasing total alkaloid concentrations (i.e. 50, 200, 350 mg total alkaloids/kg feed, respectively) for 7 weeks. The corresponding doses calculated by the CONTAM Panel are 2, 9 and 14 mg/kg bw per day, respectively. At the end of the treatment, 10 animals/group were killed and liver and kidneys were removed and subjected to gross and microscopic examination. No deaths or clinical signs of illness were detected in both trials irrespective of the alkaloid dietary intake. In experiment 1, a dose-related linear decrease in body weight gain and feed intake was detected, starting from 14 mg/kg bw per day. In experiment 2, growth rate and feed intake were depressed only at the highest dose tested (14 mg/kg bw per day); neither macroscopic nor microscopic changes were detected in liver and kidney from all treated piglets. Although no control group was included, the authors concluded that growing pigs can tolerate lupin alkaloids present in the studied L. angustifolius bitter cultivars at a dietary concentration up to 200 mg/kg. This concentration corresponds to a dose of 9 mg/kg bw per day.

A further study was conducted in Polish Large White × Pietrain barrows (average weight 53 kg) (Rotkiewicz et al., 2007). Animals were fed 62 days with diets in which the soybean meal (control diet) was replaced by lupin seeds of three L. angustifolius varieties, namely Baron, Zeus and Wersal, with slightly different total alkaloid concentrations (360, 410 and 560 mg/kg DM, respectively; analytical method not reported). The corresponding concentrations in the diet were 97, 119, 156 mg/kg diet (DM). Only in barrows offered the diet containing the lupin variety Wersal with the highest alkaloid concentration, daily weight gain was statistically significantly lower than controls (680 g vs. 740 g). In lupin-fed pigs, histopathological examinations revealed some degree of atrophy and deformation of intestinal villi and an increase in eosinophils and lymphocytes in the enteric wall. In addition, ‘proliferation of glandular and hepatic cells’ was recorded. Due to the lack of a statistical evaluation of the histopathological lesions, these findings cannot be used for hazard characterisation. Based on the reduced weight gain observed for the diet containing the highest alkaloid concentration, the CONTAM Panel concluded that pigs can tolerate lupin alkaloids present in the studied L. angustifolius varieties at a dietary concentration of 119 mg/kg. In the absence of reported feed intakes, the corresponding dose could not be calculated.

A study was designed to examine the effects of feeding two different levels of raw or germinated seeds of L. luteus (yellow lupin) or L. angustifolius (blue lupin) on zootechnical performances in growing pigs (Kasprzowicz-Potocka et al., 2013). Total alkaloid content of the Raw Yellow Lupin (RYL) seeds was 1,000 mg/kg, with sparteine and lupinine amounting to approximately 58% and 27% and low levels of lupanine (3%) and 13α-OH-lupanine (6%). Germinated Yellow Lupin (GYL) seeds had a lower total alkaloid content (700 mg/kg) with limited variation (about 10% less) in the percentages of the mentioned QAs. Total alkaloid content of the Raw Blue Lupin (RBL) seeds (1,500 mg/kg) was higher than that measured in RYL. As expected, RBL also showed a different QA composition, in that lupanine (69%) and 13α-OH-lupanine (24%) accounted for more than 90% of total QAs. Germinated Blue Lupin (GBL) seeds had a lower total alkaloid content (1,000 mg/kg) with lupanine (52%) and 13α-OH-lupanine (18%) accounting for only 70% of total QAs. Two experiments were performed, both lasting 33 days. In Experiment I, 50 crossbred piglets (initial bw 10 kg) were randomly allocated to one of the following treatment groups (N = 5 per sex and per group), namely control (no lupins), RYL, GYL, RBL and GBL. In experimental diets, 50% of the soybean meal protein content of the control group was replaced by the protein of RYL, GYL, RBL or GBL lupins. The same protocol was adopted for Experiment II, but the protein replacement rose to 75%. The resulting total alkaloid content (mg/kg diet) in Experiment I and Experiment II, was the following: RYL 17 and 24, GYL 10 and 15, RBL 31 and 47, GBL 18 and 27. Zootechnical performances (final body weight, feed intake, average daily gain and feed conversion ratio) and health status were monitored in both experiments. Blood samples were collected from 6 animals/group at the end of Experiment II only. Zootechnical performances were not affected in animals from Experiment I (50% soy protein replacement) irrespective of the lupin species (L. luteus or angustifolius) or status (raw or germinated). In Experiment II (75% soya protein replacement), feed intake was significantly depressed in RBL- or GBL fed piglets and a significant rise in serum alkaline phosphatase (~ 50%) of uncertain biological relevance was observed in RYL-fed animals. However, the CONTAM Panel noted that no significant changes were observed in ALT or AST in Experiment II for all diets tested and blood enzymes were not tested in Experiment I. Therefore, the CONTAM Panel took only the zootechnical performance into account for a conclusion regarding the tolerated concentration of QAs in feed. It is concluded that piglets tolerate lupin alkaloids present in the studied L. luteus cultivar up to a dietary concentration of
24 mg/kg (1.5 mg/kg bw per day) and lupin alkaloids present in the studied *L. angustifolius* cultivar up to 18 mg/kg (1.0 mg/kg bw per day).

### 3.1.4.3. Poultry

#### Laying hens and broiler chicks

A study was designed to determine the lethality of total and selected QAs from a bitter variety of *L. albus* (cv. not mentioned) in two breeds of 10-day-old laying hens (ISAbrown and SHAVERcross) (Cubillos et al., 1999). Total alkaloids were extracted from seeds to obtain aqueous solutions (analytical technique not mentioned). Lupanine (71%) and 13α-OH-lupanine (13%) were by far the most abundant QAs in the extract, which contained less than 1% sparteine. Lupanine was purified from the total alkaloid extract and sparteine sulfate (192 mg/mL) was commercially obtained. Animals were gavaged and mortality recorded for 48 h. In ISAbrown pouls, calculated LD<sub>50</sub> (mean and 95% confidence interval) were 958 mg/kg bw (854–1,070) for total alkaloids, 1,131 mg/kg bw (929–1,378) for lupanine, and 655 mg/kg bw (509–856) for sparteine base. In SHAVERcross pouls, similar LD<sub>50</sub> values were found for total alkaloids and lupanine, being, respectively, 961 mg/kg bw (890–1,035) and 1,271 mg/kg bw (1,027–1,573), while much lower values were calculated for sparteine, i.e. 425 mg/kg bw (303–544), pointing to the occurrence of breed-related differences in the sensitivity to certain QAs. Results from this study also confirm the higher toxicity of sparteine compared to lupanine (two- to three-fold) observed in mammals.

Vogt et al. (1987) fed laying hens (age 24 weeks) with diets containing no lupins or diets containing 16% debittered lupin grit (from either *L. albus*<sup>cv</sup> P, or *L. albus*<sup>cv</sup> G or *L. mutabilis*) that contained 1.1, 6.2, 9.6 or 1.2 mg total alkaloids/kg diet, respectively, for 168 days. Total alkaloid concentration was determined with GC (no details given); according to the authors, almost 90% of the measured total alkaloid was lupanine. Using a starting weight of 1.505 kg for the animals and a mean feed intake of 116, 120, 121 and 109 g per day, respectively, a corresponding intake of 0.08, 0.49, 0.77 and 0.09 mg alkaloids/kg bw per day could be calculated. Feeding of lupin grit did not adversely affect feed intake/efficiency, laying rate, egg weight, and egg fertility/hatchability. However, the Panel noted that the applied doses are low and not expected to cause any effects.

The adverse effects of dietary lupins were assessed in 1-day-old broiler chicks (unspecified breed) offered a diet including soybean meal (control) or raw (40%, group A), dehulled (40%, group B), or autoclaved (35% group C) *L. angustifolius* (cv. Troll) seed meals, respectively, for 21 days. The total alkaloid concentration, measured by a titration method according to Ruiz (1977), was less than 100 mg/kg in the lupin seeds, which approximately corresponded to less than 40, 40 and 35 mg alkaloid/kg feed for group A, B, and C, respectively. During the first week, two chicks from group A died and further two were euthanatised in the remaining weeks showing bradycardia, twisting of the neck, leg weakness and coma. Post-mortem examination did not reveal significant gross changes. Skeletal deformities (limb deformity, crooked sternum and scoliosis) affected a total of seven individuals (3 from group A, 3 from group B, 1 from group C). In all treated groups, body weight gain, feed consumption, and feed to gain ratio were remarkably depressed, while the liver CYP level doubled. The authors concluded that in broilers the repeated dietary exposure to certain varieties of lupins may entail severe systemic effects (including skeletal deformities) which were not counteracted by dehulling or autoclaving (Olkowski et al., 2001). The CONTAM Panel noted the observed effects; however, due to the uncertainty regarding the concentration of total alkaloids in the feed, this study cannot be used for dose-response assessment.

A study was designed to investigate the effects on zootechnical performance of lupin seeds (Olver and Jonker, 1997). A SL (*L. albus* cv. Hanti), a BL (*L. angustifolius*) and soaked micronised bitter lupin (SMBL) seeds were analysed for alkaloids by titration (method according to Ruiz, 1977) and found to contain < 100 mg alkaloids/kg for the SL, < 200 mg alkaloids/kg for the SMBL and > 900 mg/kg for the BL. As a result, the soaking procedures reduced the alkaloid concentration to less than 200 mg/kg lupin seeds. The trial was conducted on male Ross 1-day-old broilers chicks which were offered for 6 weeks a control (no lupin), a SL, a BL or a SMBL diet, containing 0, 50, 100, 200, 300 or 400 g/kg of the lupin under test. In chicks fed SLs, body weight gain, feed consumption, and feed to gain ratio were almost superimposable to control values irrespective of the added lupin amount. This was also the case for carcass characteristics (moisture, fat, protein, and ash content). By contrast, all BL-fed chicks (both BL and SMBL at all dosages) showed a depression in body weight gain, feed consumption and feed to gain ratio. An increased moisture and carcass fat content were detected at the two highest lupin concentrations for the BL group and at the highest lupin concentration for the SMBL.
group, respectively. The CONTAM Panel noted the observed effects; however, due to the uncertainty regarding the concentration of total alkaloids in the feed, this study cannot be used for dose-response assessment.

Feeding experiments with *L. albus* (cv. Ultra) seeds at several levels and different processing methods were performed in Single Comb White Leghorn laying hens (Watkins and Mirosh, 1987). The lupin alkaloid concentration (GC-MS) of the seeds was 80 mg/kg and only lupanine was quantified. Raw, autoclaved (121°C for 30 min) or extruded (80°C for 20 sec) lupins were included in the diets at different percentages (10, 15, 20, 25 or 30%) and administered for up to 32 weeks. A group fed with a basal diet (no lupins) was always included. The tested parameters were mortality, feed consumption, egg production and egg weight. No differences were observed between hens offered the basal diet or diets containing autoclaved lupins (up to 20% for 12 weeks) or extruded lupins (up to 25% for 16 weeks). Only hens administered with 25 or 30% raw lupins and for the longest duration (32 weeks) showed significant reduction in egg weight and/or egg production. The authors concluded that the *L. albus* (cv. Ultra) may be fed to laying hens as a protein source at levels of up to 20% (corresponding to a lupanine concentration of 16 mg/kg feed and a daily dose of 0.9 mg/kg bw based on a body weight of 2 kg and a daily feed intake of 0.113 kg/hen).

**Turkeys**

The tolerance to the dietary inclusion of different levels of seeds of white lupins (*L. albus* cv. Ultra) was investigated in growing male and female Large White (Nicholas) turkeys (Halvorson et al., 1988). The control diet consisted of corn and soybean; in lupin-treated animals, the control diets were partially replaced by ground whole (WL) or ground dehulled (DWL) seeds. Total alkaloid content (analytical method: GC-FID) amounted to 960 mg/kg (including 740 mg/kg ‘OH-lupanine’ and 150 mg/kg lupanine) in WL seeds and to 430 mg/kg (including 190 mg/kg ‘OH-lupanine’ and 130 mg/kg lupanine) in DWL seeds used in Experiment 1, and 430 mg/kg (including 180 mg/kg ‘OH-lupanine’ and 140 mg/kg lupanine) for WL seeds used in Experiment 2. In Experiment 1, one-day old males were offered one of the following diets for 21 days: control, WL at 20% or 40% inclusion or DWL at 20% or 40% inclusion. The CONTAM Panel calculated the corresponding doses of 29, 61, 13 and 26 mg lupin alkaloids/kg bw. Tested parameters were body weight, average daily gain, average feed intake, and feed efficiency. In Experiment 2, one-day old females were assigned to one of the following groups: control diet, control diet + WL (2:1, ~33% lupins), control diet + WL (1:1, 50% lupins), control diet + WL (0.6:1, ~43% lupins). At the end of the study (17 weeks), animals were killed and organs (liver, heart, pancreas, kidney, gizzard, intestine) were removed and weighed.

In Experiment 1 (3-week feeding), there was a very similar daily feed intake in controls and all treated animals; body weight and daily weight gain were depressed only in male turkeys administered with the 40% WL diet, corresponding to a total alkaloid dose of 61 mg/kg bw per day. In Experiment 2 (17-week feeding), mortality was not affected by the treatment and the macroscopic examination of the organs did not reveal any alteration; a very slight (up to 5%) but statistically significant reduction in body weight and average daily gain was detected in turkey hens from all treated groups along with an increase in gizzard and pancreas weight. The treated groups were exposed to concentrations ranging from 61 to 215 mg/kg feed and the corresponding doses ranged from 2.7 to 9 mg/kg bw per day. According to the authors it is doubtful that the observed effects could be related to the alkaloid contents. Based on the obtained results, the CONTAM Panel could not identify a tolerated dose from this study since effects were observed at all doses tested in the experiment with the longest and lowest exposure.

**Ducks**

A study was designed in Peking ducklings (Olver and Jonker, 1998) using the same experimental design of a study performed in broilers, as described above (Olver and Jonker, 1997). The tested lupin seeds included SL (*L. albus* cv. Hanti), a BL (*L. angustifolius*) and SMBLs, with an alkaloid concentration (measured by titration) of <100 mg alkaloids/kg for the SL, >900 mg/kg for the BL and <200 mg/kg for the SMBL. Two hundred and forty ducklings were allocated to 16 groups and offered for 6 weeks one of the following diets containing 0, 50, 100, 200, 300, 400 g/kg of the lupins under test. Three animals per group were killed at the end of the treatment for carcass traits analysis (moisture, fat, protein, and ash content). Productive parameters (body weight gain, feed consumption, 29 The effect of processing on the lupin alkaloid concentration was not reported.

30 Position not indicated in the paper, but likely to be 13α-OH-lupanine.
and feed to gain ratio) were recorded after 3 and 6 weeks. Animals fed either with the SL variety or with the SMBL, did not show statistically significant differences with controls in any of the tested parameters irrespective of the amount added to the diet. By contrast, BL did not affect productive traits only at inclusion levels up to 100 g/kg diet and carcass characteristics up to 300 g/kg. According to the authors, the apparent higher sensitivity of ducklings to BLs compared to broilers might be due to a more developed sense of taste in ducks, as assessed by a higher number of taste buds (200) with respect to broilers (20) (Kare and Ficken, 1963 as cited in Olver and Jonker, 1998). The CONTAM Panel noted the observed effects; however, due to the uncertainty regarding the concentration of total alkaloids in the feed, this study cannot be used for dose-response assessment.

Japanese quails

One-day-old quail chicks (average weight 7 g, breed not reported) were randomly divided into four groups, namely a control group I and three further groups fed 15% *L. albus* seeds as follows: raw I, processed (debittered) according to a traditional Turkish method (TTM) or subjected to extrusion I. The lupin alkaloid concentration was determined by GC-FID. The trial lasted 6 weeks and feed consumption, live weight, and feed conversion efficiency were monitored weekly. Lupanine (28,000 mg/kg) accounted for more than 80% of the lupin alkaloid content in raw lupin seeds (29,000 mg/kg) and low levels of sparteine (210 mg/kg), lupinine (15 mg/kg), cytisine (185 mg/kg) and anagyrine (115 mg/kg) were also reported. While TTM proved extremely efficacious in reducing the alkaloid concentration (lupanine concentration around 400 mg/kg, all other alkaloids not detectable), the extrusion method brought about only a negligible reduction (10%) of alkaloids. While live weight and feed conversion efficiency were lower in both R and E groups up to the fourth week of treatment, at the end of the trial there were no statistically significant differences in any of the tested parameters. It was concluded that growing performances of Japanese quails are not affected by the dietary inclusion of 15% lupin seeds from *L. albus* containing up to 29,000 mg alkaloids/kg seed (corresponding to 4,771 mg lupin alkaloids/kg feed DM or 826 mg/kg bw per day) (Arslan and Seker, 2002).

3.1.4.4. Rabbits

New Zealand White rabbits (initial average weight ~ 1 kg) were allocated to three experimental groups (Battaglini et al., 1991). Each group was offered a diet containing three different levels (0, 8 or 16%) of three different cultivars of *L. albus* seeds, namely Italian (I), Australian (A) or Australian extruded (AE) cultivars, for 80 days. Total alkaloid content was measured with an ‘official EC method’ and amounted to 5.8 (I), 5.4 (A), and 1.6 (AE) g/kg DM; the three inclusion levels resulted in the following total alkaloid content in the feed (mg/kg DM): 0, 460, 930 (I), 0,430, 860 (A) and 0, 130, 260 (AE). The corresponding doses for group I were 0, 30 and 60 mg/kg bw per day, for group A 0, 30 and 55 and for group AE 0, 10 and 19. The trial lasted 80 days, but zootechnical performances were recorded from day 45 until day 80 only. As compared to controls, in the I group, only the highest inclusion level (16%) negatively affected feed consumption, final weight and daily weight gain. In rabbits of the A group exposed to the same level (16%, same total alkaloid content) a reduced feed consumption was observed. Zootechnical parameters were not affected in rabbits receiving extruded lupins (AE). From this study, it is concluded that rabbits tolerate lupin alkaloids present in the studied *L. albus* cultivars at a dose of 30 mg/kg bw per day.

3.1.4.5. Fish

Rainbow trout (*Oncorhynchus mykiss*)

A feeding study (Serrano et al., 2011) was carried out in rainbow trout to investigate the effects of lupinine, the main QA in European *L. luteus* cultivars (Wink et al., 1995), accounting for almost 60% to the total alkaloid concentration in the plant. Rainbow trout (average weight 330 ± 20 g) were allocated to 8 groups receiving a diet containing 0, 50, 75, 100, 250, 500, 1,000 or 5,000 mg lupinine/kg, respectively, for 60 days. From the data provided by the authors regarding feed intake and body weight, the CONTAM Panel calculated the corresponding doses (0, 0.5, 0.8, 1.1, 2.5, 4.5 and 7.2 mg/kg bw). A 20% and 33% reduction in feed intake was detected at 500 and 1,000 mg/kg inclusion levels, respectively; the same dosages resulted in a 30% and 50% fall in weight gain, respectively. After 40 days, the treatment with the highest concentration (5,000 mg lupinine/kg) was stopped for animal welfare reasons (extremely low feed intake and weight gain). At the end of the experimental period, 9 individuals/group were sacrificed. Body, liver and spleen were weighed. Treatment effects on productive
parameters, whole body composition analysis and tissue histopathology were also investigated. There was a dose-related quadratic depression in both feed intake and growth rate, negative effects being observed at doses greater than 1.1 mg/kg bw. Feed efficiency ratio was not affected. No relevant histopathological changes were detected, except for a depletion of glycogen and lipid stores in the 7.2 mg/kg bw dose group. Splenosomatic index was not affected, whereas decrease in the hepatosomatic index was noticed at lupinine doses higher than 1.1 mg/kg bw. It is concluded that a dose of 1.1 mg lupinine/kg bw is tolerated by rainbow trout and can be considered as a NOAEL for lupinine in rainbow trout, while 2.5 mg/kg bw can be considered as a lowest-observed-adverse-effect level (LOAEL).

A further study was conducted in rainbow trout to investigate the effects of sparteine (Serrano et al., 2012). Juvenile rainbow trout (average weight 61 ± 7 g) were allocated to 8 groups receiving a diet containing 0, 50, 100, 250, 500, 1,000, 2,500 or 5,000 mg sparteine/kg, respectively, for 62 days. From the data provided by the authors regarding feed intake and body weight, the CONTAM Panel calculated the corresponding doses (0, 0.7, 1.4, 3.5, 6.6, 11.9, 14.5 and 15.6 mg/kg bw). No mortality occurred. Final body weight, weight gain, feed consumption and hepatosomatic index were decreased in fish exposed to >1.4 mg/kg bw. By contrast, treated fish did not display relevant histopathological changes in liver, kidney, spleen, and intestine, suggesting that the adverse effects of sparteine in rainbow trout are likely to reflect the decrease in feed intake. In conclusion, doses of sparteine up to 1.4 mg/kg bw per day are safe in trout and this dose can be considered as a NOAEL for sparteine in rainbow trout. The LOAEL identified from this study was 3.5 mg/kg bw.

3.1.4.6. Horses

No information about QA toxicity in horses was identified.

3.1.4.7. Companion animals

One study in cats and dogs was identified, which is described in Section 3.1.2.3.

3.1.4.8. Summary remarks

Limited data are available regarding the adverse effects caused by QAs in farm animals, horses and companion animals. Most studies used lupin seeds as source of QAs in the diet and do not report the QAs present in the diet or their relative contribution to the total QA concentration. Such studies do not allow identification of NOAEL/LOAELs. These studies, as well as studies that allowed the identification of NOAEL/LOAELs are summarised in Appendix H.6

In cattle, only studies on zootechnical performance were identified and from these, the CONTAM Panel identified 50 mg/kg bw per day as a dose of lupanine and 13α-OH-lupanine present in seeds from L. albus that can be tolerated. No relevant studies were identified for sheep and goats.

Case reports on lupin toxicosis are only reported for pigs, indicating that pigs are sensitive to QAs. Based on zootechnical performance, the CONTAM Panel identified 1-10 mg/kg bw per day as a dose range of lupin alkaloids present in seeds from L. angustifolius and 1.5 mg/kg bw per day as a dose of lupin alkaloids present in seeds from L. luteus that can be tolerated by pigs. In addition to an effect on zootechnical performance, gastro-intestinal lesions have been observed in pigs receiving a lupin seed-based diet. However, due to the lack of a statistical evaluation, these findings could not be taken into account for identifying tolerated doses.

An acute toxicity study in laying hens, confirms the higher toxicity of sparteine compared to lupanine observed in mammals. Based on zootechnical performance, 1 mg/kg bw per day was identified as a dose of lupin alkaloids present in seeds from L. albus that can be tolerated by laying hens. In a study with one-day-old broiler chicks (for which no dose calculation was possible), effects on zootechnical performance, as well as severe systemic effects, were observed, however at concentrations in the feed that were higher compared to the studies in laying hens. No tolerated dose could be identified for turkeys since effects were observed on zootechnical performance at the lowest dose tested of 2.7 mg/kg bw per day. In one-day-old quail chicks, 826 mg/kg bw was identified as a dose of lupin alkaloids present in seeds from L. albus that can be tolerated (only dose tested).

For rabbits, the CONTAM Panel identified 30 mg/kg bw per day as a dose of lupin alkaloids present in seeds from L. albus that can be tolerated.

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31 The organ weight expressed as percentage of body weight.
Pure lupinine and sparteine were tested in feeding studies with rainbow trout, and NOAELs of 1.1 and 1.4 mg/kg bw per day were identified for lupinine and sparteine, respectively, for the effect on zootechnical performance and hepatosomatic index.

3.1.5. Mode of action

Typically, human acute poisoning cases involving intake of QAs from lupin seeds present symptoms consistent with the so-called anticholinergic syndrome with symptoms associated with the central nervous system, the gastro-intestinal system and the cardiovascular system. Classically these include mydriasis, dry mucous membranes, reduced saliva production, tachycardia and/or arrhythmia, urine retention, confusion and general malaise. Respiratory paralyses have also been reported. Suggested modes of action for these effects are interaction with ACh receptors both in the CNS and in the peripheral autonomic system, involving both nicotinic ACh receptors (nAChR) in ganglions and peripheral mAChR of postganglionic parasympathetic nerve fibres. The nAChR and mAChR are two classes of acetylcholine receptors. There are two main subtype groups of the nicotinic receptors, N1 called peripheral or muscular (motor endplates) receptor subtypes and the N2 subtypes known as the central or neuronal receptor. The nAChRs consist of five subunits. In humans, there are 16 different subunits encoded by separate genes, and the nAChRs are both homomeric and heteromeric. The nAChR is coupled to non-selective cation channels and, dependent on subunit composition it transports sodium, potassium and in some instances calcium (Colquhoun et al., 2003; Zoli et al., 2018). The mAChR form G protein-coupled receptor complexes in the cell membranes of certain neurons, i.e. in postganglionic fibres in the parasympathetic nervous system. There are five subtypes of the muscarinic receptors (M1-5). Separate genes encode these subtypes, which are expressed differently in the tissues (Carlson and Kraus, 2018). There are also polymorphisms in both nAChR and mAChR (Michel and Teitsma, 2012; Deflorio et al., 2017).

Acute symptoms of lethal amounts of QAs given orally are similar in rats, mice and guinea pigs, with clinical signs showing shaking, excitation and convulsions and death resulting from respiratory failure. In cases with respiratory paralysis, also nAChR (subtype N1) of the motor endplates of the muscles might have been affected. Mild intoxicated cows exhibited mild signs of uncoordinated gait, muscle fasciculations or coarse muscle tremors, and increased sensitivity to external stimuli. The effects in experimental animals and cattle as described above are also compatible with QAs interacting with cholinergic receptors.

Yovo et al. (1984) examined sparteine and lupanine in an in vitro binding assay with ACh receptors isolated from rat brain. Binding to the receptors was assayed using displacement of 3H-nicotine and 3H-quinuclidinyl benzilate. The authors concluded that sparteine and lupanine had a very weak affinity for the mAChR with Ki32 of 7 and 11 μM, respectively, whereas the respective values for the nAChR were 0.4 and 0.5 μM. The authors noted the selectivity of both compounds for the nicotinic sites. This accords with the ganglioplegic effects observed for both compounds in cats and dogs (Yovo et al., 1984; see also Section 3.1.2.3 Neurotoxicity).

Voitenko and co-workers (1991) investigated the effect of (+)-sparteine on nAChR in neurons in rat superior cervical ganglion of the sympathetic system. They found that (+)-sparteine (2 μM) reduced ACh induced activation in a competitive manner. Also, an open-channel-blocking of (+)-sparteine was observed, but this effect was relatively low, possible due to the larger size of the molecule relative to channel profile.

Cytisine has been tested in experimental systems and has been found to possess nAChR agonist activity, being a partial agonist of α4/β2* nAChRs and a full agonist at α3/β4* nAChRs (Luetje and Patrick, 1991; Picciotto et al., 1995; Mineur et al., 2007; Li et al., 2010).

Schmeller et al. (1994) further examined the inhibitory action of the major QAs present in L. albus, L. mutabilis, L. luteus, L. angustifolius, Anagyris foetida and Laburnum anagyroides at the nAChR and mAChR. They used ACh receptor preparations from porcine brain and measured QA induced displacement of 3H-nicotine and 3H-quinuclidinyl benzilate. From the resulting displacement curves, half maximal inhibitory concentration (IC50) values were calculated which are presented in Table 3. The affinities for the receptors differed from those found by Yovo et al. (1984) who used rat brain instead of pig brain as source of receptor preparation.

32 The Ki (inhibitor constant) is an indication of how potent an inhibitor is. It is the concentration required to produce half maximum inhibition.
From the study of Schmeller et al. (1994), it can be concluded that various QAs bind to both ACh receptors, but with different affinity. For example, lupanine appears to have the strongest affinity to the nAChR. Hydroxylation of lupanine strongly reduces its affinity to the nAChR. N-Methylcytisine and cytisine seem to have the strongest affinity to the nAChR and 13α-tigloyloxylupanine for the mAChR. These binding studies represent the neuronal N2 nAChR subtypes and a mixture of all five mAChR subtypes. Any impact of polymorphic variants of the receptors for the QAs is unknown. Contrary to the finding of Yovo et al. (1984), Schmeller et al. (1994) found that sparteine preferentially binds to the mAChR, however different species, rats and pigs were used in these receptor binding studies. The CONTAM Panel further noted that interaction with cholinergic receptors is in agreement with toxicity observed in the CNS affecting cholinergic pathways following QA exposure (see Section 3.1.2.3 Neurotoxicity). While receptor affinity could give an indication of relative toxic potencies of the different QAs, it is not known how the QAs might affect different receptor subtypes and whether toxic potency in vivo would also depend on kinetic differences, e.g. in peak plasma concentration and elimination speed. Although both Yovo et al. (1984) and Schmeller et al. (1994) used receptor preparations from brain, it is unclear whether the difference in relative affinity to the receptors is related to species differences or other assay conditions.

Green and co-workers examined whether sparteine, lupanine and anagyrine could bind to and cause desensitisation of muscle-type nAChR and turning it into a state that closes the cation channel. As this would ultimately result in inhibition of foetal movements, desensitisation has been suggested as a mode of action for teratogenicity in cattle induced by lupin alkaloids containing anagyrine (Green et al., 2017). Using cell culture models expressing either autonomic ganglion-type nAChR or fetal human muscle-type nAChR, the authors found that lupanine or sparteine had no desensitising effects, whereas anagyrine appeared to be a strong nAChR desensitiser. As anagyrine is not relevant for the current Scientific Opinion, it is therefore not further discussed in this section.

In addition to the interaction of QAs with neuroreceptors, i.e. the ACh receptor, it has also been assumed based on electrophysiological studies that QAs and in particular sparteine affects sodium inward current thereby reducing excitability of muscles and nervous tissues and affecting cardiac action potentials. It is well known that voltage dependent ion channels are responsible for the excitability of nerve, heart smooth muscle and skeletal muscle cells.

Sparteine and lupanine inhibit both sodium and potassium channels (Galvan et al., 1984; Honerjager et al., 1986) and there are several reports on the impact of QAs on such channels. Pugsley et al. (1995) investigated the electrophysiological effects on rat heart and isolated rat myocard cells with patch-clamp technique. Sparteine prolonged the P-R and WaT intervals and reduced the

| Compound             | IC₅₀ (a) nAChR (µM) | IC₅₀ mAChR (µM) |
|----------------------|---------------------|-----------------|
| Albine               | 193                 | 33              |
| Anagyrine            | 2,096               | 132             |
| Angustifoline        | > 500               | 25              |
| Cytisine             | 0.14                | 400             |
| 3β-OH-Lupanine(b)    | 190                 | 74              |
| 13α-OH-Lupanine(b)   | 490                 | 140             |
| Lupanine             | 5                   | 114             |
| Lupinine             | > 500               | 190             |
| N-Methylcytisine     | 0.05                | 417             |
| Multiflorine         | > 500               | 47              |
| 17-Oxosparteine      | 155                 | 118             |
| Sparteine            | 331                 | 21              |
| Tetracydrooniumbifenine | 310           | 129             |
| 13α-Tigloyloxylupanine | 160             | 11              |

mAChR: muscarinic acetylcholine receptor; nAChR: nicotinic acetylcholine receptor.
(a): The concentration that displaces 50% of the specifically bound radiolabelled ligand.
(b): The CONTAM Panel noted inconsistency in the reporting of the stereochemistry by the authors. However, based on the available reports on QA composition of lupin seeds, the CONTAM Panel assumed that the compounds tested are 3β-OH-lupanine and 13α-OH-lupanine.
similarly or less active than sparteine. Scarce data on other modes of action of a few QAs might indicate that these QAs are ajmaline\(^33\) and 55.7 \(\mu\)M for sparteine, 6.6 \(\mu\)M for ajmaline\(^33\) and 55.7 \(\mu\)M for quinidine.\(^33\) Sparteine had about 1/20 and lupanine about 1/100 of the activity of ajmaline, the most potent Na\(^+\) channel inhibitor. Again, it is noted that the relative effects of these compounds in \(\text{in vivo}\) also depend on the kinetic properties.

Sparteine also causes blockade of the calcium-activated potassium channels (OMOTE et al., 1993).

In addition, other possible modes of action of QAs have been investigated.

Wink (2004) reviewed the biological functions and molecular modes of action of QA and, in addition to the effects of QAs described above, found that minor cellular targets of QAs could be dopamine, \(\gamma\)-aminobutyric acid (GABA), \(N\)-methyl-D-aspartate (NMDA) and alpha 2 receptors.

In an attempt to identify new inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BchE), sparteine and cytisine were tested in \(\text{in vitro}\). Cytisine was completely inactive against both enzymes. The anti-AChE effect of sparteine was modest (< 50%) \(EC_{41.6} = 1 \, \mu\)g/mL\(^\text{a}\), while an appreciable inhibition towards BchE (> 70%) \(EC_{33.9} = 1 \, mg/mL\) was observed (Orhan et al., 2007).

Sparteine was introduced in the late 1930s to induce labour and the uterotonic effect of QAs has been investigated in old studies using \(\text{in vitro}\) set-ups with uterine preparations from both experimental animals and humans (Garg et al., 1973). Ligon (1941) used \(\text{in vitro}\) uterine segments from rabbits to investigate the impact of sparteine sulfate, sparteine disulfate, lupinine base, \(d\)-lupanine dihydrochloride and trilupine base.\(^34\) They found that the sparteine compounds were the most potent followed by lupinine, which was five times less potent, and trilupine and \(d\)-lupanine, which were 10 to 15 times less potent than sparteine. Garg et al. (1973) treated strips of both non-pregnant and pregnant human uterus in \(\text{in vitro}\) (5-20 \(\mu\)g/mL of sparteine sulfate) and found that response on contractions was dose-related with pregnant uterus about 10 times more sensitive than non-pregnant uterus. Spasms were observed at the highest doses (20 \(\mu\)g/mL of sparteine sulfate). The uterotonic effects of chemicals may be mediated by several mechanisms (Gruber and O’Brien, 2011). One such mechanism is via the prostaglandin pathway, which was investigated in the rat by Abtahi et al. (1978). In \(\text{in vivo}\) experiments, they found that sparteine (10 mg/kg bw) increased the prostaglandin F concentration in plasma and uterine tissue of pregnant, but not of non-pregnant rats. Indomethacin, a well-known inhibitor of prostaglandin synthesis abolished the effect of sparteine. Indomethacin pretreatment (5-10 \(\mu\)g/mL) strongly reduced the effect of sparteine in \(\text{in vitro}\) experiments using uterine segments from pregnant rats treated with sparteine (80-3,220 \(\mu\)g/mL) aiming to give between 80% and 90% of maximum contraction. Indomethacin did not have any effect of prostaglandin F2\(\alpha\) or Acetylcholine induced contractions.

Negative results were reported for the ability of several QAs to inhibit protein synthesis (13\(\alpha\)-OH-lupanine and sparteine), or bind RNA (anagyrine, angustifoline, cytisine, 3\(\beta\)-OH-lupanine, 13\(\alpha\)-OH-lupanine, lupinine, lupinone, 17-oxosparteine, sparteine, 13\(\alpha\)-tigloyloxylupanine) (Korcz et al., 1987; Wink et al., 1998; Merschjohann et al., 2001; Lin et al., 2011).

**Summary remarks**

Several modes of action have been identified for QAs, the most important in relation to acute toxicity being their binding and inhibitory action on AChRs producing an anticholinergic syndrome of toxicity with paralysis of respiratory muscles causing respiratory failure and death. The different QAs appear to bind with different affinity to these receptors and also their preference for nAChRs or mAChRs seems to differ. QAs may also cause, with different potencies, inhibition of voltage dependent ion channels, e.g. sodium and potassium channels, and calcium activated potassium channel, effects possibly underlying their antiarrhythmic effect. The uterotonic effects of sparteine appears to be mediated via an increased production of prostaglandin F. No studies investigating mode of action and possible additive effect of QAs were identified. Most data are on sparteine. With regard to the other QAs relevant in this opinion, affinities to AChRs appear to be in the same range or lower than those of sparteine. Scarce data on other modes of action of a few QAs might indicate that these QAs are similarly or less active than sparteine.

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\(^33\) An alkaloid (non-QA) that is a well-known sodium channel inhibitor and used as an antiarrhythmic agent.

\(^34\) Trilupine was shown to be \(d\)-lupanine monohydrochloride dihydrate (see Marion, 1952).
3.1.6. Consideration of critical effects and dose–response analysis

3.1.6.1. Acute toxicity

Experimental animal data

The acute toxicity of lupanine was two- to fourfold higher in mice compared with rats when delivered either by i.p. (LD$_{50}$: 80 vs. 177 mg/kg bw, respectively) or orally (LD$_{50}$: 410 vs. 1,664 mg/kg bw, respectively). Studies in rats indicated that acute toxicity of lupanine and 13$_{\alpha}$-OH-lupanine is similar (LD$_{50}$: 177 and 199 mg/kg bw by i.p., respectively). Sparteine is two times more toxic than lupanine in mice (oral LD$_{50}$: 220 vs. 410 mg/kg bw, respectively) as well as in guinea pigs. Following oral intakes of lupanine, 13$_{\alpha}$-OH-lupanine and sparteine, the clinical symptoms of poisoning were similar in all species, with the cause of death being identified in respiratory failure and anticholinergic symptoms. The similarity of the symptoms of intoxication supports the conclusion on a similar mode of action of these QAs.

Human data

Lupin seeds

Typical symptoms of acute intoxication in human with lupin seeds are associated with their content of bitter QAs and are usually described as anticholinergic syndrome, often also affecting the cardiovascular and digestive systems. Death is usually due to respiratory failure (see Section 3.1.5). In poisoning cases with lupin seeds, the estimated total alkaloid intakes range from about 10 to 50 mg/kg bw. Reports suggest that children are more susceptible to intoxication with lupin alkaloids than adults and that doses from 10 mg/kg bw onwards have to be considered to be lethal in children.

Sparteine

For sparteine sulfate,$^{35}$ antimyrrhythmic properties have been reported in a single oral dose of as low as 20 mg (for a person with a body weight of 70 kg equivalent to 0.29 mg sparteine sulfate/kg bw or 0.16 mg sparteine/kg bw) (Thies, 1986; Blaschek et al., 2006). According to clinical experiences, only higher single doses of 150–200 mg (equivalent to 2.1–2.9 mg/kg bw for a person with a body weight of 70 kg) could ensure a reliable antimyrrhythmic effect. For short-term use, the recommended daily dosage for (-)-sparteine sulfate ranged from 800 to 1,000 mg per day (equivalent to 11.4–14.3 mg/kg bw for a person with a body weight of 70 kg), being divided into 4–5 single doses of 200 mg (equivalent to 2.9 mg/kg bw for a person with a body weight of 70 kg) (Rote Liste, 1983). Individual variations in sensitivity to sparteine sulfate were due to the genetic polymorphism, which the isoenzyme CYP2D6 underlies (Eichelbaum et al., 1986; Thies, 1986). At these dosages, PMs were considered as a group at higher risk to develop adverse effects, which include gastrointestinal disturbances, central nervous disorders, dizziness, head pressure, accommodation disturbances in the eye, bradycardia, negative inotropy, hypertonica, changes in haemogram and liver enzymes. Use of sparteine sulfate as an antimyrrhythmic drug during pregnancy was, because of its uterotonic effect, considered contraindicated (Rote Liste, 1983; Thies, 1986; Blaschek et al., 2006; Aktories et al., 2009).

Sparteine sulfate also showed oxytocic activity and was effective in reducing the first stage of labour when given in oral single doses of 100–150 mg (equivalent to 1.4–2.1 mg/kg bw for a person with a body weight of 70 kg) by repeating dosage every hour if needed, up to a total dose of 600 mg (equivalent to 8.6 mg/kg bw for a person with a body weight of 70 kg). An adverse effect reported for the pregnant women was vomiting (Dipont, 1971).

At oral doses from 40 mg/kg bw onwards sparteine blocks the nAChR (N1 subtype) of the motoric endplate of muscles, which may lead to respiratory failure and death. In cases of intoxication, where peripheral respiratory paralysis has been overcome by artificial ventilation, higher oral doses from 90 mg sparteine/kg bw onwards, induce bradycardia with the risks of fatal outcome due to cardiac arrest (Thies, 1986; Blaschek et al., 2006; Aktories et al., 2009). Accidental ingestion of approximately 30 mg of sparteine/kg bw was fatal to a young child (Schmidt, 1961).

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$^{35}$ According to Milne (2018) and The Merck Index (2006), it is assumed that sparteine sulfate is used in the form of the pentahydrate (MW: 422.54). A single dose of 20 mg sparteine sulfate pentahydrate (equivalent to 0.29 mg/kg bw for a person with a bw of 70 kg) would then correspond to a single dose of 11.09 mg sparteine (MW: 234.38) (equivalent to 0.16 mg/kg bw for a person with a bw of 70 kg).
Lupanine, 13α-OH-lupanine

In a kinetic study, no adverse effects were observed after administration of single oral doses of 10 mg of lupanine or 10 mg 13α-OH-lupanine (each equivalent to 0.14 mg/kg bw for a person with a body weight of 70 kg) to volunteers (n = 11) either classed as EMs or PMs (Petterson et al., 1994).

Livestock and companion animal data

In the only relevant study, oral LD₅₀ of total alkaloids from a bitter variety of *L. albus*, lupanine and sparteine was determined in 10-day-old laying hens of two different breeds (ISAbrown and SHAVERcross). In ISAbrown poults, calculated LD₅₀ (mean and 95%) were 958 mg/kg bw (854–1,070) for total alkaloids, 1,131 mg/kg bw (929–1,378) for lupanine, and 655 mg/kg bw (509–856) for sparteine. In SHAVERcross LD₅₀ for total alkaloids and lupanine were 961 mg/kg bw (890–1,035) and 1,271 mg/kg bw (1,027–1,573), respectively, for lupanine, while for sparteine an LD₅₀ of 425 mg/kg bw (303–544) was calculated. Results from this study confirm the higher toxicity of sparteine compared to lupanine observed in mice and guinea pigs and indicate possible breed-related differences in the sensitivity to certain QAs.

Overall summary for acute toxicity

Comparison of doses reported from human intoxication with those resulting in acute toxicity in experimental animals shows that humans are more sensitive to QAs than laboratory rodents. The observed difference in acute toxicity might be linked to the differences in toxicokinetics between humans and rats and particularly the biotransformation of sparteine to lupanine in the rat, which is not reported to occur in humans. Therefore, the CONTAM Panel used the human data as a basis for hazard characterisation in the assessment of human risks due to exposure with QAs via food.

Supported by human data on sparteine, the CONTAM Panel concluded that anticholinergic and antiarrhythmic effects of QAs are the critical acute effects for the hazard characterisation. No human studies are available describing the dose-response relationship for these effects of QAs.

Knowledge on dose-responses refers only to oral therapeutic doses of sparteine sulfate used in the treatment of cardiac arrhythmia, which is mainly due to the effect on the voltage dependant sodium channels. Adverse effects associated with this treatment belong to the spectrum of the anticholinergic syndrome. A dose of 20 mg of sparteine sulfate (equivalent to 0.16 mg sparteine/kg bw assuming a body weight of 70 kg) is described as the lowest single oral dose with antiarrhythmic properties, which is one-fifth of the lowest single oral dose of sparteine sulfate associated with oxytocic activity.

Only one study in poultry was identified that supports the conclusion from experimental animals that sparteine is more toxic than lupanine. No other data in livestock or companion animals were identified.

3.1.6.2. Repeated-dose toxicity

Experimental animal data

All the repeated dose studies with laboratory animals on QAs were carried out in rats. Single dose, sub-chronic studies on diets containing *L. albus*-, *L. luteus*-, and *L. mutabilis* seeds did not identify significant toxicological effects. Two studies investigated a diet containing increasing concentrations of *L. angustifolius* flour. The major QAs in the flour were lupanine and 13α-OH-lupanine. Both studies reported a slight increase in relative liver weights. However, some conflicting results were reported in different publications from the same laboratory.

Due to several reasons including differences in sources of QAs in feeds, control diets and types of cultivars, the CONTAM Panel concluded that the repeated-dose studies in experimental animals have a limited value for the evaluation of QA toxicity.

Human data

In the long-term therapy of cardiac arrhythmias the recommended daily dosage for (-)-sparteine sulfate was half of that for short-term treatment and ranges from 400 to 500 mg (equivalent to 5.7 to 7.1 mg/kg bw for a person with a body weight of 70 kg), divided into 4–5 single doses of 100 mg (equivalent to 1.4 mg/kg bw for a person with a body weight of 70 kg). Known adverse effects and contraindications were the same as for short-term use indicated above (Rote Liste, 1983).

No other human data on adverse effects associated with long-term exposure to lupin seeds or QAs occurring in lupin seeds are available. The CONTAM Panel concluded that the available data are too limited to identify a reference point for the human risk assessment following repeated exposure.
Livestock and companion animal data

The large majority of relevant repeated-dose studies in livestock species (except fish, see below) were performed with the sole aim of identifying the lupin dietary inclusion levels not affecting zootechnical performances (feed intake, weight gain, feed efficiency, milk yield, egg production). The CONTAM Panel noted the considerable differences in both total and specific QA content among lupin seeds from different species and even cultivars as well as the lack of specific toxicity studies. Therefore, based on the available data, NOAEL/LOAEL for most food producing species could not be identified and were replaced by suggested tolerated concentrations/doses specifically referring to seeds from a single lupin species/cultivar. The only relevant studies performed with pure QAs (i.e. lupinine and sparteine) were conducted in rainbow trout and allowed to identify similar NOAEL values for lupinine and sparteine (1 mg/kg bw per day). In the absence of data on other QAs, the CONTAM Panel used this NOAEL for a group approach to characterise the hazard of the QAs relevant for this opinion and applied to all farmed salmonids. No relevant studies have been identified for companion animals.

3.1.7. Possibilities for derivation of a human health-based guidance value

Based on the available information, the CONTAM Panel concluded that anticholinergic effects and the electrical conductivity of the heart following acute exposure to QAs are the critical effects for the human hazard characterisation. The mode of action of sparteine and its pharmacological profile as an anticholinergic substance are considered similar to those of other main alkaloids in lupin seeds, such as lupanine, 13α-OH-lupanine and lupinine. Therefore, the CONTAM Panel considers it appropriate to use a group approach and apply the principle of dose-additivity for the combined activity of these QAs acting on the same target receptors. Based on the findings of studies on acute toxicity in experimental animals, in vitro studies showing inhibition of ion channels, in particular sodium channels, and effects on the isolated uterus, sparteine is assumed to have a higher potency than other QAs from lupin seeds. Therefore, and in the absence of dose-response information for other QAs, the CONTAM Panel decided to base the hazard characterisation on sparteine as a conservative approach. Although sparteine is only described as one of the major alkaloids in the seeds of *L. luteus* and *L. mutabilis* (see Table 1), the approach is also considered valid for seeds of *L. albus* and *L. angustifolius*.

The available data were considered insufficient to propose HBGVs and instead an MOE approach using the lowest single oral antiarrhythmic dose of 0.16 mg sparteine/kg bw as reference point (see Section 3.1.6.1) was selected to characterise the risk following acute exposure since the CONTAM Panel considers that an intended pharmacological effect following therapeutic use is an unwanted effect in the context of risk assessment for food. No reference point could be identified to characterise the risk following chronic exposure.

3.2. Occurrence data

3.2.1. Current occurrence data submitted to EFSA

Data were collected according to the procedure described in Section 2.2. Occurrence data on QAs for the present assessment were provided by one national authority from the Netherlands and one University from Italy.36

An initial number of 1,465 analytical results on 13α-OH-lupanine, 13α-angeloyloxylupanine, 13α-tiglioyloxylupanine, albine, angustifoline, α-isolupanine, lupanine, lupinine, multilorine, sparteine, tetrahydrohombifoline in feed (n = 390) and food (n = 1,075) were present in the EFSA Chemical Occurrence database.

After the validation processes (Section 2.2.2), data providers were invited to clarify possible inconsistencies identified by EFSA’s DATA Unit. Where such a clarification was not received from the data provider, the CONTAM Panel was required to apply certain assumptions.

It should be noted that when reference is made to lupins in this section, as well as in the Annexes, this should be understood as lupin seeds.

The CONTAM Panel is aware that both whole and dehulled seeds are used for food and feed. The proportion of seed coat to whole seed is variable depending on the variety but may account for up to 25% of the weight of the whole seed (Brand et al., 2004). However, no studies have been identified in

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36 University of Milano – Dipartimento di Scienze Farmaceutiche – DISFARM.
which the levels of QAs, and their proportions, have been determined separately in the seed coat and in the kernel (dehulled seed). Therefore, in this exposure assessment it has been assumed that levels and proportions are similar in the whole and dehulled seeds, but recognised that if the levels or proportions are different this would result in an under- or overestimation of the exposure.

3.2.1.1. Occurrence data on food

A total of 1,035 analytical results on 99 food samples were retained after data cleaning. All samples referred to lupin-based foods. Details of the data cleaning applied for food samples are presented in Annex A (Table A.2).

Analytical results were submitted by the Netherlands (540 analytical results from 54 samples on 10 QAs) and by the University of Milan (495 analytical results from 45 samples on 11 QAs); detailed information on the number of samples versus analysed substance, sampling year and reporting country/institution can be retrieved in Table A.3 of Annex A. The sampling year spanned from 2005 until 2018, with most of the samples collected in 2016 (Annex A, Table A.3).

Table 4 provides an overview of the number of samples per food category according to different levels of the FoodEx2 classification system.

| FoodEx2 Category (Level 4)                      | FoodEx2 Category (Level 6)                           | N  |
|------------------------------------------------|-----------------------------------------------------|----|
| Almonds and similar-                           | Almonds(1)                                          | 1  |
| Bread and rolls with special ingredients added | Bread and rolls with special ingredients added       | 1  |
| Canned or jarred legumes                       | Canned or jarred legumes                             | 8  |
| Coffee imitate ingredients                    | Coffee imitate ingredients                           | 4  |
| Isolated proteins and other protein products   | Isolated proteins and other protein products         | 6  |
| Lupins (dry) and similar-                      | Blue lupin (dry)                                    | 27 |
|                                                 | Lupins (dry)                                        | 9  |
|                                                 | White lupin (dry)                                   | 29 |
| Meat imitates                                  | Meat imitates                                       | 8  |
| Pasta based dishes, cooked                     | Pasta, plain (not stuffed), cooked                  | 1  |
| Pasta, plain (not stuffed), uncooked           | Dried durum pasta                                   | 3  |
| Pasta-like products                            | Pasta-like products                                 | 1  |
| Rusk                                           | Rusk                                                | 1  |

N: number of samples.
(1): Almond flour containing a certain amount of lupin flour.

The vast majority of occurrence data were reported for samples (n = 65) classified as ‘Lupins (dry) and similar’. This type of samples represents lupin seeds that are sold as dry seeds and that can be consumed upon rehydration, debittering, boiling and brining or as flour that can be used for homemade dishes (such as pancakes/crepes or pasta). Information on the lupin species was reported in some cases: 29 samples were classified as L. albus and 27 as L. angustifolius. Twenty-eight samples of ‘Lupins (dry) and similar-’ were reported to be processed (i.e. ’grinding/milling/crushing’) and the results therefore refer to lupin seeds in the form of flour or grits. Samples reported in the category ‘canned or jarred legumes’ referred to lupin seeds in water and salt or pickled (those are normally consumed as such). In the category ‘canned or jarred legumes’, all samples were left-censored for lupinine, tetrahydrodrombifoline and albine, while for lupanine, multiflorine and 13α-OH-lupanine only 13% of the samples were left-censored. In the category ‘Lupins (dry) and similar-‘, left-censored results were as follows: lupanine 0%, 13α-OH-lupanine 13%, angustifoline 11% and α-isolupanine 18%. Details on the percentage of left-censored data in food samples can be found in Table A.4 (Annex A).

Under the category ‘meat imitates’ different types of lupin morsels, steaks or cutlets were reported, in few cases the % of lupin flour was reported as 40%. For lupanine and 13α-angeloyloxy-lupanine, no left-censored samples were present in this category.

In the category ‘Pasta-like products’, pasta to which isolated protein was added as ingredient was reported, while in the category ‘Pasta, plain (not stuffed), uncooked’ lupin flour was added as ingredient.

Fifty-four samples were analysed with LC-MS/MS and an LOQ of 0.1 mg/kg was reported for all tested substances (i.e. 13α-OH-lupanine, 13α-angeloyloxy-lupanine, 13α-tigloyloxy-lupanine,
angustifoline, α-isolupanine, lupanine, lupinine, multiflorine, sparteine, tetrahydrorhombifoline). The other samples were analysed with GC-MS and an LOQ of 1.5 mg/kg was reported for all tested substances (i.e. the substances analysed by LC-MS/MS plus albine).

In the category ‘canned or jarred legumes’, all samples were left-censored for lupinine, tetrahydrorhombifoline and albine, while for lupanine, multiflorine and 13α-OH-lupanine only 13% of the samples were left-censored. In the category ‘Lupins (dry) and similar’, left-censored results were as follows: lupanine 0%, 13α-OH-lupanine 13%, angustifoline 11% and α-isolupanine 18%. Full details on the percentage of left-censored data in food samples can be found in Table A.4 (Annex A).

Descriptive statistics by food category for lupanine and TotQA are presented in Table 5 while the full descriptive statistics for all the QAs can be found in Table A.5 (Annex A). TotQA is calculated as the sum of the 6 most abundant QAs in the data set available, namely lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine and α-isolupanine.

Very high levels of lupanine (up to 5,395 mg/kg) and TotQAs were found in certain samples belonging to the category ‘Lupins (dry) and similar’. For this reason, a clarification request was sent to the data provider. It was confirmed that a part of the reported samples (n = 22) were not present on the EU market but rather cultivated for research purposes. These research samples showed a higher mean concentration of lupanine compared to the other samples of lupin seeds (768 vs 192 mg/kg LB = UB), multiflorine (60 vs 11 mg/kg LB) and 13α-angeloyloxylupanine (27 vs 2.6 mg/kg LB), but showed a lower mean concentration of 13α-OH-lupanine, 13α-tigloyloxylupanine and angustifoline. Based on this information, the CONTAM Panel decided to exclude the research samples from the data set used for dietary exposure assessment.

Table 5: Descriptive statistics of lupanine and total quinolizidine alkaloids concentrations (mg/kg) by food category

| FoodEx2 category (Level 4) | N  | LB/UB | Lupanine | TotQA(a) |
|---------------------------|----|-------|----------|----------|
|                           |    |       | Min      | Mean     | Max      | Min     | Mean     | Max     |
| Almonds and similar-(b)   | 1  | LB    | 24       |          |          | 53      |          |         |
|                           |    | UB    | 24       |          |          | 53      |          |         |
| Bread and rolls with special ingredients added | 1 | LB    | 8.4      |          |          | 21      |          |         |
|                           |    | UB    | 8.4      |          |          | 21      |          |         |
| Canned or jarred legumes  | 8  | LB    | 0.0      | 16       | 54       | 0.0     | 25       | 75      |
|                           |    | UB    | 1.5      | 16       | 54       | 4.2     | 26       | 75      |
| Coffee imitate ingredients| 4  | LB    | 98       | 165      | 238      | 186     | 331      | 482     |
|                           |    | UB    | 98       | 165      | 238      | 186     | 331      | 482     |
| Isolated proteins and other protein products | 6 | LB    | 8.5      | 29       | 67       | 8.5     | 29       | 67      |
|                           |    | UB    | 8.5      | 29       | 67       | 16      | 37       | 74      |
| Lupins (dry) and similar- (research) | 22 | LB    | 26       | 768      | 5,395    | 26      | 922      | 6,100   |
|                           |    | UB    | 26       | 768      | 5,395    | 34      | 926      | 6,105   |
| Lupins (dry) and similar- | 43 | LB    | 8.9      | 191      | 1,859    | 25      | 429      | 3,673   |
|                           |    | UB    | 8.9      | 191      | 1,859    | 25      | 429      | 3,676   |
| Meat imitates             | 8  | LB    | 7.9      | 22       | 40       | 15      | 28       | 46      |
|                           |    | UB    | 7.9      | 22       | 40       | 19      | 33       | 52      |
| Pasta based dishes, cooked| 1  | LB    | 3.0      |          |          | 6.5     |          |         |
|                           |    | UB    | 3.0      |          |          | 6.8     |          |         |
| Pasta, plain (not stuffed), uncooked | 3 | LB    | 0.0      | 3.9      | 11       | 0.0     | 9.0      | 26      |
|                           |    | UB    | 0.1      | 3.9      | 11       | 0.6     | 9.3      | 26      |
| Pasta-like products       | 1  | LB    | 2.7      |          |          | 10      |          |         |
|                           |    | UB    | 2.7      |          |          | 10      |          |         |
| Rusk                      | 1  | LB    | 4.2      |          |          | 4.2     |          |         |
|                           |    | UB    | 4.2      |          |          | 12      |          |         |

LB: lower bound; Max: maximum; Min: minimum; N: number of samples; TotQA: total quinolizidine alkaloids; UB: upper bound.

(a): Calculated as the sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine, α-isolupanine.

(b): Almond flour containing a certain amount of lupin flour.

(c): Only one sample available in the data set and the concentration of this sample is reported under the mean concentration.
3.2.1.2. Occurrence data on feed

A total of 390 analytical results on QAs were reported on 37 feed samples. Details of data cleaning are reported in Table B.1 (Annex B).

Analytical data on feed were submitted by the Netherlands (170 analytical results from 17 samples on 10 QAs) and by the University of Milan (220 analytical results from 20 samples on 11 QAs); the sampling year spanned from 2011 until 2018, with most of the analytical results from samples collected in 2017. Detailed information can be retrieved in Table B.2 (Annex B).

The University of Milan submitted 5 samples coded as 'Legume seeds and products derived thereof (feed)' and 15 as 'Lupin middlings (feed)'. It should be noted that some of the samples reported in the category 'Legume seeds and products derived thereof (feed)' were specified by the data provider as 'mixtures of dry legume seeds containing also dry lupin seeds (from L. albus) although with % lupin and composition unknown'. The category 'Lupin middlings (feed)' includes samples specified as 'lupin flour for pig feed with percentage of lupin and composition unknown'. The Netherlands submitted 17 samples of 'sweet lupins (feed)', which were all L. angustifolius seeds.

Both data providers reported that several samples were suspect. All 20 samples of 'Lupin middlings (feed)' and 'Legume seeds and products derived thereof (feed)' reported by the University of Milan were collected from pig farms where intoxication had been reported. In the category named as 'sweet lupins (feed)'; 6 suspect samples were reported as well. A description of the number of feed samples and percentage of left-censored data per substance can be found in Table B.3 of Annex B.

The 17 samples reported by the Netherlands were analysed using LC-MS/MS for 13α-OH-lupanine, 13α-angeloxyloxylupanine, 13α-tigloyloxylupanine, angustifoline, α-isolupanine, lupanine, lupinine, multiflorine, sparteine, tetrahydrorhombifoline. The LOQ was 0.1 mg/kg for all QAs reported. The 20 samples reported by the University of Milan were analysed by GC-MS for the 10 substances mentioned above plus albine; all were reported with an LOQ of 1.5 mg/kg.

Descriptive statistics of the occurrence data for feed samples reported in this opinion can be found in Table 6. As for the food, the CONTAM Panel decided to focus on lupanine and TotQA as the sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine and α-isolupanine. More detailed descriptive statistics for all the alkaloids can be found in Table B.4 (Annex B).

Table 6: Descriptive statistics of lupanine and total quinolizidine alkaloids concentrations (mg/kg) by feed category

| Category(a) | N | Suspect | LB/UB | Lupanine | TotQA(b) |
|-------------|---|---------|-------|----------|----------|
|             |   |         |       | Min      | Mean     | Max      | Min      | Mean     | Max      |
| Legume seeds and products derived thereof (feed) | 5 | YES | LB | 479 | 3,307 | 6,164 | 517 | 3,834 | 6,896 |
| (based on L. albus) | | | UB | 479 | 3,307 | 6,164 | 522 | 3,839 | 6,901 |
| Lupin middlings (feed) | 15 | YES | LB | 0.0 | 514 | 1,070 | 0.0 | 611 | 1,201 |
| (based on L. albus) | | | UB | 1.5 | 514 | 1,070 | 9.0 | 616 | 1,206 |
| Sweet lupins (feed) | 11 | NO | LB | 19 | 102 | 265 | 58 | 257 | 559 |
| (L. angustifolius) | | | UB | 19 | 102 | 265 | 58 | 257 | 559 |
| Sweet lupins (feed) | 6 | YES | UB | 100 | 3,553 | 7,459 | 396 | 9,571 | 19,004 |

LB: lower bound; Max: maximum; Min: minimum; N: number of samples; TotQA: total quinolizidine alkaloids; UB: upper bound.
(a): based on the FoodEx2 classification system supplemented with information on species provided by the data providers.
(b): calculated as the sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine, α-isolupanine.

Due to the large differences in mean concentrations between suspect and non-suspect samples, the CONTAM Panel decided not to use feed samples reported as suspect (Table 6) for the exposure assessment for farm animals, horses and companion animals. Consequently, a very limited number of samples (n = 11) were available for the exposure assessment. The CONTAM Panel therefore decided to complement the data set with data on occurrence of QAs in 'Lupin (dry) and similar'-reported as food samples for which the species was reported (13 of L. albus and 21 of L. angustifolius), plus

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37 Suspect sampling strategy is defined as 'Selection of an individual product or establishment in order to confirm or reject a suspicion of non-conformity. It is a not random sampling. The data reported refer themselves to suspect units of the population' according to EFSA (2011).
9 samples for which the lupin species was not specified. Chemical occurrence data reported for *L. angustifolius* as food and feed were merged, while for *L. albus* only food samples were available. Table 7 shows the descriptive statistics of the final data set.

**Table 7:** Occurrence data used for exposure assessment for farm animals, horses and companion animals (mg/kg)

| Species                        | N   | Scenario | Lupanine | TotQA          |
|--------------------------------|-----|----------|----------|----------------|
|                                |     |          | Min      | Mean           | Max  | Min | Mean | P75 | Max  |
| *L. albus*                     | 13  | LB       | 29       | 166           | 959  | 68  | 344  | 359 | 1,125|
|                                |     | UB       | 29       | 166           | 959  | 71  | 345  | 359 | 1,130|
| *L. angustifolius*             | 32  | LB       | 19       | 205           | 1,859| 58  | 475  | 539 | 3,673|
|                                |     | UB       | 19       | 205           | 1,859| 58  | 476  | 539 | 3,676|
| *L. angustifolius, L. albus, and unspecified* | 54  | LB       | 8.9      | 13            | 1,859| 25  | 394  | 352 | 3,673|
|                                |     | UB       | 8.9      | 13            | 1,859| 25  | 394  | 352 | 3,673|

LB: lower bound; P75: 75th percentile; TotQA: total quinolizidine alkaloids; UB: upper bound.

### 3.2.1.3. Relative variations of main QAs in lupin seeds

Figure 5 shows the relative percentage of the 6 main QAs normalised by the sum for *L. albus* (n = 13) and *L. angustifolius* (n = 21) seeds that were reported as food samples as well as *L. angustifolius* seeds (n = 11) reported as feed samples. All suspect samples as well as the research samples were not included.

Considering the small number of samples per category, results should be considered with caution. Lupanine was the most abundant QA in both species, followed by 13α-OH-lupanine (especially abundant in *L. angustifolius*) and angustifoline. Multiflorine and 13α-tigloyloxylupanine were more abundant in *L. albus* than in *L. angustifolius*. These observations, although arising from a relatively small number of samples, are in agreement with the scientific literature (Table 1).
Figure 5: Contribution of single quinolizidine alkaloids to the total quinolizidine alkaloid concentration (sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine, α-isolupanine) in lupin seeds food samples of *L. albus* (n = 13), *L. angustifolius* (n = 21) and in lupin seeds feed samples of species *L. angustifolius* (n = 11) at the upper bound scenario. Box plots display from left to right: minimum, P25, median, P75 and maximum.
3.2.1.4. Occurrence data received during the public consultation

During the public consultation (EFSA, 2019), EFSA received analytical results from 121 samples of *L. albus* and *L. angustifolius* from a company analysed by GC. The concentration of total alkaloids ranged from 10 to 1.149 mg/kg with an average concentration of 184 mg/kg and a P90 of 368 mg/kg. The CONTAM Panel noted the lower concentration of total alkaloids in the data received during the public consultation compared to the data submitted to EFSA during the call for data. It was noted that for some of the samples only lupanine and 13-OH-lupanine were analysed and based on the assumption that these substances contribute for 85% to the total QA content, the total QA content was calculated.

3.2.2. Previously reported occurrence data in the open literature

Food

Two papers have been identified that describe the QA content in lupin-based foods.

Reinhard et al. (2006) studied six QAs as well as ammodendrine and gramine in food on the Swiss market using GC-FID and GC–MS (Table 8). Except from lupin coffee, the concentrations of alkaloids are very low compared to the concentration in seeds/flours analysed in the same study (see Appendix D, Tables D.1–D.4). However, lupin coffee is also the only food that is made solely from lupin seeds. Primarily lupanine, 13α-OH-lupanine, and angustifoline were found in the foods. The three QAs – α-isolupanine, 13α-angeloyloxylupanine and 13α-tigloyloxylupanine – were only present in trace amounts in two of the lupin coffee samples, in the curry burger, the lupin tofu products and one of the breads.

**Table 8:** Concentration of alkaloids in different lupin-based food products (Reinhard et al., 2006)

| Food product                     | Number of samples | Concentration of total alkaloids (mg/kg) |
|----------------------------------|-------------------|----------------------------------------|
| Lupin coffee                     | 4                 | 136–182                                |
| Curry burger                     | 1                 | 4                                      |
| Lupin tofu                       | 2                 | 12–15                                  |
| Lupin tofu with tomato           | 1                 | 3                                      |
| Bread (7–10% lupin flour)        | 2                 | 8–12                                   |
| Falafel with lupin tofu          | 1                 | 3                                      |
| Lupin snack                      | 1                 | 6                                      |

Resta et al. (2008) analysed eight samples of lupin-based commercial foods by GC-MS (see Table 9). The results of the total QA concentration is the sum of the found QAs and is denoted ‘estimated total alkaloids’.

**Table 9:** Concentration of total alkaloids and identified quinolizidine alkaloids in lupin-based food products as reported by Resta et al. (2008)

| Food                              | Concentration of total alkaloids (mg/kg)(a) | Quinolizidine alkaloids found                      |
|-----------------------------------|--------------------------------------------|--------------------------------------------------|
| Meat imitates (tofu, steak, morsels, cutlet, steak with mushrooms) | 15–56                                      | Albine, lupanine, 13α-angeloyloxylupanine, 13α-OH-lupanine |
| Pasta (with lupin concentrate)    | 2.7                                        | Lupanine                                          |
| Lupin rusk (with lupin flour)     | 4.2                                        | Lupanine                                          |
| ‘Lupini beans’                    | < LOQ(b)                                   |                                                  |

LOQ: Limit of quantification.
(a): Estimated as sum of quinolizidine alkaloids.
(b): Given in the paper as 2 mg/kg for lupanine in flour.

Feed

No information regarding the occurrence of QAs in whole plants (which would include stems, leaves and seeds) fed to livestock has been identified. In a few papers results from leaves are included and these results are summarised in Appendix D, Table D.6 to give an indication of the concentrations and
distribution in plant parts other than seeds for the 11 relevant QAs. The CONTAM Panel noted that in general the same QAs are found in seeds and leaves while the distribution of the individual QAs can differ.

3.2.3. Factors influencing the concentration of quinolizidine alkaloids/Food and feed processing

3.2.3.1. Debittering of lupin seeds

BL seeds need to be debittered before consumption. The traditional house-hold methods include soaking in cold water, cooking and washing and this process lasts several days (Smith, 1987; Carvajal-Larenas et al., 2014). In Ecuador, the National Agricultural Research Centre has proposed a commercial debittering process which consists of soaking the seeds of L. mutabilis for 14–16 h, boiling for 40 min and then washing at 40°C (Carvajal-Larenas et al., 2013).

Carvajal-Larenas et al. (2016) published an overview paper about debittering of L. mutabilis seeds. In a study at commercial scale, 93% of the alkaloid content was removed after treating lupin seeds with warm water (40°C) for 90 h. A process where the seeds were soaked for 14–20 h (15°C), boiled for 1/2 - 2 h and then washed for 96–120 h, removed 97% of the alkaloid content while cooking for 1/2 h and extraction with cold water for 72 h removed 95% of the alkaloid content. Also, debittering with other solutions than water has been described e.g. treatment with alkaline solutions (Carvajal-Larenas et al., 2016). According to the authors, water treatment is the only known process that is applied at commercial scale and the CONTAM Panel also considers that water treatment is the only process of interest for debittering at home.

Jimenez-Martinez et al. have published several papers (2001, 2004, 2009, 2010) about debittering of seeds of a wild variety of L. campestris. The seeds were boiled for 6 h with water or in an acid or alkaline solution. The liquid was changed every 20 min. Water treatment including boiling removed between 89% and 99% of the original QA content.

Biological processes can also be used to debitter lupin seeds and this includes bacterial fermentation, fungal fermentation and germination. According to Carvajal-Larenas et al. (2016), bacterial fermentation reduced the concentration of alkaloids by < 50%, fungal fermentations reduced the concentration with 50–91% while germination caused a reduction of 78%.

Gefrom et al. (2013) have studied the effect of ensiling on the concentration of QAs in crushed lupin seeds. A sweet (Bora) and a bitter (Azuro) cultivar of L. angustifolius were harvested at 65% dry matter content and four different kinds of model silages were prepared. The samples were stored at 20°C for 90 days and analysis was performed by GC-MS. The authors concluded that ensiling had no significant effect on the QA content.

Chilomer et al. (2010) investigated the influence of germination on the total amount of QAs as well as on single QAs. Seeds of L. angustifolius (cv. Graf) and L. luteus (cv. Lord) were soaked and washed before germination that was conducted in darkness for 48, 72 and 96 h at 15°C or 24°C. The samples were analysed by GC-NPD For both lupin varieties, the concentration decreased with time and temperature (up to 30%), so the lowest concentration was obtained after 4 days at 24°C. Also, the concentration of the different QAs differed with time and temperature.

De Cortes Sánchez et al. (2005) studied the change in QA concentration during the germination of seeds from a wild variety of L. campestris from Mexico as well as bitter seeds from L. albus L. (LO-3,923) and L. angustifolius (1,413) from Spain. Samples were taken each day until day 9 of the germination. Determination of QAs was performed by GC-NPD and the single substances were identified by GC-MS. For L. angustifolius, the total alkaloid concentration was the same at day 0 and day 9, while in seeds from L. albus and L. campestris the final concentrations were higher at day 9 than on day 0. Also, here the concentrations of the different QAs differ with time and the results for the individual QAs from day 0 are shown in Appendix D, Tables D.1 and D.2 for L. albus and L. angustifolius. The results from this study are not in accordance with the results from the paper by Chilomer et al. (2010).

A completely different process was developed by Haddad et al. (2006). Seeds of L. albus and L. mutabilis, with alkaloid concentrations of, respectively, 250 mg/kg DM and 55,000 mg/kg DM, were cracked, dehulled, moistened and treated by instantaneous controlled pressure drop technology (DIC), soaked for 2 h in water, and dried to a water content of 5%. The total alkaloid concentration was determined by GC-NPD. The total alkaloid concentration decreased by 60–70%.
3.2.3.2. Changes during production of processed food

Erbas (2010) debittered seeds from a bitter variety of *L. albus* grown in Turkey to produce a lupin snack. The process consisted of cleaning, boiling, debittering and salting. After boiling the seeds were debittered using three different processes, but only one result (mean of the three processes) was given. The alkaloids were determined by GC-FID with sparteine as internal standard. The total amount of alkaloids decreased from 14,400 mg/kg DM in the seeds to 6,200 mg/kg DM after boiling, to 10 mg/kg DM after debittering and below the LOQ in the snack (LOQ: \(\leq 7\) mg/kg). In the raw seeds besides lupanine (87%) also multiflorine (11%) and small amounts of sparteine, albine and \(\alpha\)-isolupanine were identified (see Appendix D, Tables D.1–D.4), but during the debittering process the QAs were removed gradually starting with sparteine after 12 h and ending with \(\alpha\)-isolupanine after 120 h.

Resta et al. (2008) investigated the changes in QA concentration due to food preparation. In total 16 samples of lupin protein isolates (LPI; food ingredient) and model foods were prepared (see Table 10) and analysed by GC-MS. In the protein isolates, only lupanine was identified. For the other processed foods, made using lupin flour, the authors report that the same QAs as in the seeds were found (see Appendix D, Tables D.1–D.4) but no amounts were given for the individual QAs. As can be seen from Table 10, the process from seeds to protein isolate is efficient in reducing QAs.

Table 10: Total alkaloid concentrations in seeds, protein isolates and lupin-based processed foods (Resta et al., 2008)

| Food                  | Total alkaloids (mg/kg) | Food                  | Total alkaloids (mg/kg) |
|-----------------------|-------------------------|-----------------------|-------------------------|
| *L. albus* (cv. Arès) |                         | *L. albus* (cv. Tytop) |                         |
| Seeds                 | 146                     | Seeds                 | 1,247                   |
| Beverage, 8.8% flour  | 20.7                    | LPI-E\(^{(a)}\)        | 21.4                    |
| Snacks, 40% flour (N = 3) | 78.5–86.5              | Spaghetti, 5% LPI-E   | < LOQ                   |
| Biscuit, 7.5% flour   | 50.5                    | Spaghetti, 10% LPI-E  | < LOQ                   |
| Biscuit, 15% flour    | 83.9                    | LPI-M\(^{(a)}\)        | 66.5                    |
| LPI-E\(^{(a)}\)       | 9.0                     | LPI-F\(^{(a)}\)        | 8.45                    |
| Biscuit, 6.75% LPI-E  | < LOQ                   | *L. angustifolius* (cv. Boregine) |
| Biscuit, 13.5% LPI-E  | 3.1                     | Seeds                 | 1,107                   |
| LPI-F\(^{(a)}\)       | 37.0                    | LPI-E\(^{(a)}\)        | 34.1                    |

LOQ: limit of quantification; LPI: lupin protein isolate.\(^{(a)}\): Different preparations of lupin protein isolates.

Based on the available information, the CONTAM Panel concluded that between 88 and 97% of the QAs present in seeds are removed by water treatment and boiling. Studies of the influence of germination on the QA concentrations show no clear effect.

3.3. Exposure assessment

It should be noted that when reference is made to lupins in this section, as well as in the Annexes, this should be understood as lupin seeds.

3.3.1. Current dietary exposure assessment for humans

Given the limited amount of data available in the EFSA occurrence database for most food groups, as well as limited data on consumption of lupin seeds and lupin-based foods, the CONTAM Panel decided to calculate acute dietary exposure for some specific food groups under specific scenarios.

3.3.1.1. Occurrence data and scenarios definition

Occurrence levels used to calculate acute dietary exposure in the different scenarios are summarised in Table 11.

Scenario 1 assumes that a consumer purchased lupin seeds and prepared them at home, upon no application of a debittering procedure or of a completely ineffective debittering procedure. In this scenario, it is therefore assumed that no reduction of the QA content is obtained. Occurrence results on samples reported in the category ‘Lupins (dry) and similar’ (\(n = 34\)) were used. However, based on
the information provided, it was not possible to discriminate between sweet and bitter varieties. The CONTAM Panel decided to calculate dietary exposure using both the mean occurrence value of TotQA (429 mg/kg) and the highest reliable percentile (P90) to represent exposure from lupin seeds with average and high QA content.

In scenario 2, it is assumed that the lupin seeds are debittered by soaking and boiling in water and a reduction factor of 89% to the concentration of TotQA is applied (see Section 3.2.3). Occurrence results reported for ‘Lupins (dry) and similar’ (n = 34) were used but upon application of an 89% reduction factor. As for scenario 1, exposure was calculated both for mean and P90 to represent exposure from lupin seeds with average and high QA content.

In scenario 3, levels of TotQA in ‘Lupin seeds as canned or jarred legumes’ were used. It is assumed that the consumer buys pre-prepared lupin seeds such as the one in brine. In this case, the number of samples (n = 8) was insufficient to calculate a reliable percentile, therefore only the mean TotQA at the LB and UB was used for exposure calculations.

Finally, scenario 4 was elaborated to simulate the exposure to QAs via the consumption of meat imitates. The mean TotQA concentration was used to calculated dietary exposure at the LB and UB. Only 8 samples were available and therefore also it was not possible to calculate a high percentile.

The CONTAM Panel considered the possibility to develop scenarios reflecting the exposure to QAs from lupin flour as an ingredient in bread and pasta and from coffee imitates. However, the number of samples on lupin-based bread, pasta and coffee imitates was too limited to calculate dietary exposure. In addition, the uncertainty regarding the inclusion percentage of lupin flour in bread and pasta was too high to develop a scenario.

### 3.3.1.2. Consumption

The CONTAM Panel estimated acute dietary exposure to TotQA as defined in Section 2.2.2 from lupin and lupin-based products.

The latest version of the EFSA Comprehensive Database (Annex A.1) was browsed for all the FoodEx2 categories containing lupins as a main constituent or as an ingredient. The consumption of ‘Lupins seeds without pods’ was reported only in a limited number of consumption days (63 for adults and less than 10 subjects in all the other population groups) in the Portuguese population. Consumption statistics in grams per day and grams per body weight per day are reported in Annex C.1. Mean and the high percentile (as the 95th) of acute consumption (in one day) were calculated for adults only as 0.8 and 1.8 g/kg bw per day, corresponding to 57 and 142 g/day of lupin seeds, respectively. It is noticeable that the portion size used by the BfR (2017a) to carry out the exposure assessment on the German population was 100 g and therefore can be considered in line with data extracted from the EFSA Consumption Database. Mean and high consumption values in adults for ‘Lupin seeds without pods’ were used for scenarios 1, 2 and 3.

The CONTAM Panel used the consumption of ‘Meat imitates’ retrieved in the EFSA Comprehensive Database as a proxy for meat imitates based on lupin seeds. The consumption across different population groups in 17 Member States is reported in Annex C.2. The largest consumers at the 95th percentile (based on more than 60 days of consumption) were the Swedish adults with a consumption of 6.9 g/kg bw per day corresponding to 330 g/day. Individual consumption data of ‘Meat imitates’ retrieved in EFSA comprehensive database were used for Scenario 4.

Table 11: Description of the scenarios and occurrence values used in this opinion to assess acute exposure to TotQAs

| Scenario | Mean TotQA concentration (mg/kg)(b) | High TotQA concentration (mg/kg) | Consumption data used in this scenario |
|----------|-------------------------------------|----------------------------------|----------------------------------------|
| 1 | Consumption of lupin seeds with no reduction of QA content | 429 | 958 (P90) | P95 consumption in adults (Portugal only) of ‘Lupins (without pods)’ |
| 2 | Consumption of lupin seeds with 89% reduction of QA content | 47 | 105 (P90) | P95 consumption in adults (Portugal only) of ‘Lupins (without pods)’ |
| 3 | Consumption of jarred lupin seeds in brine | 25–26 | NA(a) | P95 consumption in adults (Portugal only) of ‘Lupins (without pods)’ |
3.3.1.3. Results

Results of the current dietary exposure assessment for humans are reported in Annex C, and P95 dietary exposures are summarised in Table 12.

Under scenario 1, the 95th percentile of acute exposure was 761 µg/kg bw based on lupin seeds with average content of TotQA and 1,700 µg/kg bw for lupin seeds with high content of TotQA. Upon application of a debittering procedure (scenario 2) leading to an 89% reduction of exposure, the P95 acute exposure was 84 and 187 µg/kg bw for lupin seeds with average and high content of TotQA, respectively. Under scenario 3, where canned lupin seeds were considered as source of acute exposure to QAs, the mean exposure was 20–21 µg/kg bw and the P95 44–46 µg/kg bw (LB–UB). It should be noted that these scenarios reflect the exposure of adults, but also other age groups may consume lupin seeds. However, due to limitations in the consumption data, no scenarios for other age groups, specifically children, were calculated.

In addition, according to scenario 4, consumption of lupin-based meat imitates (Annex C.2), the P95 highest exposure was assessed to be 194–228 µg/kg bw (LB–UB) for adults from Sweden.

Table 12: High exposure (P95) to TotQA upon consumption of lupin seeds or lupin-based products under the five exposure scenarios

| Scenario | Consumption Data Used in this Scenario |
|----------|----------------------------------------|
| 4 | Consumption of lupin-based meat imitates |
| Mean TotQA concentration (mg/kg)(b) | High TotQA concentration (mg/kg) |
| NA(a) | 28-33 |

NA: not applicable; P90/95: 90/95th percentile; TotQA: total quinolizidine alkaloids.
(a): Not enough observations to calculate a reliable percentile.
(b): In case lower bound (LB) and upper bound (UB) values were identical, only one value is shown, otherwise the LB-UB range is shown.

3.3.2. Current dietary exposure assessment for farm animals, horses and companion animals

Although the Lupinus genus is a widely diverse family of plants, only four species are commercially cultivated as grain legume crops (L. angustifolius, L. luteus, L. albus and L. mutabilis). Of these, seeds (grains) of L. angustifolius are the most commonly used as feeds for livestock (Frick et al., 2017).

Lupins are grown both for their seeds, which are used in food and feed, and for forage. Lupin seeds contain high levels of protein and oil and may be used to replace soybean meal and other oilseed meals in the diets of livestock and aquaculture species (Lucas et al., 2015). White lupin seeds are predominantly used as feed for livestock and are normally flaked or milled before feeding. The crop may also be grazed or cut for ensiling or used as a green manure. However, no occurrence data are available for QAs in whole crop or ensiled lupins, and therefore, no exposure from these feeds has been possible.
Lupin seeds contain an outer coat (or hull) which may account for 20–30% of the weight of the seed. For ruminants, the whole seed may be fed, although the seeds are usually ground before feeding to high yielding dairy cows. For non-ruminant species and fish, the outer coat is removed to improve digestibility; nevertheless, inclusion rates of kernel meal into pig and poultry diets are still limited by the presence of non-starch polysaccharides in the kernel.

In common with other legume seeds, lupin seeds are deficient in sulfur-containing amino acids, which also restricts their use for non-ruminant livestock, fish and companion animal, although this deficiency may be overcome by supplementation with synthetic amino acids in non-organic diets.

In the absence of any internationally recognised limits on the inclusion rates of lupin seed meal in the diets of livestock and fish, the CONTAM Panel have used maximum inclusion levels recommended in a number of scientific publications, details of which are given in Appendix G.2. Similarly, assumptions made for feed intake and physiological state of individual species are given in Appendix G.1. These data, together with the levels of the QAs in lupin seeds (Table 7), have been used to estimate daily exposure to QAs by the individual animal species. In doing so, the Panel acknowledges that the estimated exposure levels are likely to represent the maximum – or worst-case – exposure, rather than ‘typical’ exposures.

Estimates of mean LB dietary exposure for farm animals, horses and companion animals to TotQAs based on occurrence data for lupin seeds (see Table 7) are presented in Table 13. The UB dietary exposure is very similar to the LB, and differences between the mean and P75 dietary exposure were also generally small. These exposures are shown in Appendix G.

### Table 13: Estimates of mean lower bound exposure (mg/kg bw per day) to TotQAs\(^{(a)}\) from lupin seeds for farm animals, horses and companion animals

| Farm animal | All lupin seeds (n = 54) | L. angustifolius (n = 32) | L. albus (n = 13) |
|-------------|-------------------------|--------------------------|-------------------|
| Dairy: high yielding | 1.00 | 1.21 | 0.88 |
| Beef: fattening | 0.35 | 0.43 | 0.31 |
| Sheep: lactating | 1.03 | 1.25 | 0.90 |
| Goats: lactating | 3.34 | 4.04 | 2.92 |
| Goats: fattening | 1.18 | 1.43 | 1.03 |
| Horses | 0.39 | 0.48 | 0.34 |
| Pig starter | 2.95 | 3.56 | 2.58 |
| Pig finisher | 2.95 | 3.56 | 2.58 |
| Lactating sow | 2.36 | 2.85 | 2.06 |
| Chickens for fattening | 3.54 | 4.28 | 3.10 |
| Laying hens | 3.54 | 4.28 | 3.10 |
| Turkeys for fattening | 1.97 | 2.38 | 1.72 |
| Ducks for fattening | 2.75 | 3.33 | 2.41 |
| Salmonids | 1.57 | 1.90 | 1.38 |
| Cats | 0.59 | 0.71 | 0.52 |
| Dogs | 0.57 | 0.68 | 0.50 |
| Rabbits | 4.43 | 5.35 | 3.87 |

bw: body weight; TotQA: total quinolizidine alkaloids.
\(^{(a)}\): Calculated as the sum of lupanine, 13\(-\)OH-lupanine, angustifoline, multiflorine, 13\(-\)tigloyloxylnupaine, \(\alpha\)-isolupanine.

### 3.4. Risk characterisation

It should be noted that this risk assessment relates to lupin seeds and lupin-derived products with a QA profile comparable with that of the submitted data (see Section 3.2.1) and should not be extrapolated to lupin seeds with a different QA profile (e.g. lupin seeds from species and/or varieties high in sparteine).

#### 3.4.1. Human health risk characterisation

The available data were considered insufficient to propose HBGVs and instead an MOE approach is used. The CONTAM Panel selected the lowest described single oral antiarrhythmic dose of 160 µg sparteine/kg bw in humans as reference point to characterise the risk following acute exposure. No
reference point for chronic exposure could be identified due to the limited data available. The CONTAM Panel decided to calculate acute dietary exposure only for a few food groups under four specific scenarios because of the limited occurrence and consumption data. Consequently, no full risk characterisation was possible. Certain lupin-based foods such as pasta, bread and coffee imitates could not be included and exposure of children was not taken into consideration for scenarios 1, 2 and 3. In addition, insufficient occurrence data were available for scenario 3 and 4 to allow calculation of the acute dietary exposure based on the highest reliable percentile for the occurrence and only the mean occurrence value was used.

There is uncertainty linked to the reference point of 160 $\mu$g sparteine/kg bw (see Section 3.5.4). This reference point for QAs is based on the effects of sparteine which is the most potent QA with respect to toxicity. However, sparteine occurs only in trace amounts according to the data submitted to EFSA and, according to clinical experience, doses 7.5–10 times higher than the reference point ensure a reliable antiarrhythmic effect. Therefore, the CONTAM Panel concluded that MOEs $> 1$ do not indicate a health concern.

Dietary exposure to QAs from lupin seed consumption was calculated using 3 scenarios for adults only (Table 12). Comparison of this dietary exposure to the reference point of 160 $\mu$g/kg bw per day, results in MOE values that are presented in Table 14. A first scenario, assuming that a consumer is purchasing dry lupin seeds and does not debitter the seeds, resulted in MOE values below 1. The calculated exposures for scenario 1 are within the dose-range reported for sparteine to have therapeutic antiarrhythmic effects, or a possible oxytocic effect on the pregnant uterus. The calculated MOEs for scenario 1 indicate that lack of debittering may result in a risk for the consumer. It was noted that calculated exposure may reach nearly 20% of the doses of total alkaloids that have been reported to be lethal in children.

In scenario 2, it was assumed that the seeds are debittered and a reduction factor of 89% was applied. The calculated MOEs were 0.9 (high QA concentration) and 1.9 (average QA concentration). For the consumption of lupin seeds with a high QA concentration that are debittered, the MOE is below 1, which may indicate a health concern.

In scenario 3, dietary exposure to QAs from ready-to-eat lupin seeds was calculated and the corresponding MOE was about 3.5. Due to the low number of samples for this scenario, no calculation could be made of dietary exposure following the consumption of lupin seeds high in QA content.

In scenario 4, dietary exposure from lupin-based meat imitates, was calculated and the MOE value was 0.8–0.7 (Table 14). However, it should be noted that the number of samples is small.

Table 14: Margin of exposure (MOE) values for acute effects from consumption of lupin seeds or lupin-based products under the four exposure scenarios

| Scenario                                      | P95 exposure (µg TotQA/kg bw per day) | MOE calculated from P95 acute exposure |
|-----------------------------------------------|--------------------------------------|----------------------------------------|
| 1 Consumption of lupin seeds with no reduction (Mean QA content) | 761(a) | 0.2(a) |
| 1 Consumption of lupin seeds with no reduction (High QA content) | 1,700(a) | 0.1(a) |
| 2 Consumption of lupin seeds with 89% reduction (Mean QA content) | 84(a) | 1.9(a) |
| 2 Consumption of lupin seeds with 89% reduction (High QA content) | 187(a) | 0.9(a) |
| 3 Consumption of jarred lupin seeds in brine | 44–46 (LB-UB)(a) | 3.6–3.5 (LB-UB)(a) |
| 4 Consumption of lupin-based meat imitates | 194–228 (LB-UB)(b) | 0.8–0.7 (LB-UB)(b) |

LB: lower bound; P95: 95th percentile; QA: quinolizidine alkaloids; UB: upper bound.
(a): Based on Portuguese adult population only.
(b): Based on the most exposed population at the P95 (Adults from Sweden) according to the present assessment.

3.4.2 Animal health risk characterisation

Dietary exposure to QAs from lupin seeds was calculated for different livestock species (Table 13). In the absence of any feed industry data on levels of lupin seeds in livestock feeds in the EU, the CONTAM Panel has used maximum recommended inclusion levels (See Appendix G). Therefore, levels
of exposure calculated in this opinion represent worst-case scenarios. It should be mentioned that insufficient data on levels of QAs in lupin seeds were available to allow P95 estimates of exposure to be made. Instead P75 estimates of exposure were calculated, but since these were broadly similar to the mean dietary exposure, they have not been included in the risk characterisation. Furthermore, no data were available to estimate potential exposure to QAs from forage lupins (fresh or conserved) consumed by ruminants.

For salmonids, the CONTAM Panel applied a group approach and used a NOAEL of 1 mg/kg bw per day for the relevant QAs in this opinion (see Section 3.1.6). The mean dietary exposure of salmonids to TotQA was 1.6 mg/kg bw per day, which is above the NOAEL but below the LOAEL of 2.5 mg/kg bw per day. The CONTAM Panel considers the risk for adverse effects in salmonids to be low.

For the other species, the available database on adverse effects was too limited to identify overall NOAEL/LOAELs. Instead, as an alternative approach, the CONTAM Panel identified doses of QAs present in seeds from *L. luteus*, *L. albus* and *L. angustifolius* that can be tolerated with respect to zootechnical performance. However, such a dose is only applicable to lupin seeds with similar QA composition as used in the study from which the dose was derived, and does not allow a full characterisation of the risk. For horses and companion animals, no reliable data were identified that allowed the identification of such doses.

For cattle, 50 mg/kg bw per day was identified as a dose of the sum of lupanine and 13α-OH-lupanine present in seeds from *L. albus* that can be tolerated. The mean dietary exposure of cattle to TotQAs from *L. albus* ranges from 0.3 to 0.9 mg/kg bw per day, which is well below the tolerated dose.

The mean dietary exposure of pigs to TotQAs in seeds from *L. angustifolius* ranged from 2.9 to 3.6 mg/kg bw per day, which is within the dose range of 1-10 mg/kg bw per day that can be tolerated by pigs.

For chickens, 1 mg/kg bw per day was identified as a dose of lupin alkaloids present in seeds from *L. albus* that can be tolerated. The mean dietary exposure of chickens to TotQAs from *L. albus* is 3.1 mg/kg bw per day, which is higher than the tolerated dose. For Japanese quails, a tolerated dose of 826 mg/kg bw per day was identified but no estimation of dietary exposure was possible for this species.

The mean dietary exposure of rabbits to TotQAs in seeds from *L. albus* was 3.9 mg/kg bw per day, which is well below the dose of 30 mg/kg bw per day that can be tolerated by rabbits.

### 3.5. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to QAs in lupin seeds and lupin-based food has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2007). In addition, the report on ‘Characterizing and Communicating Uncertainty in Exposure Assessment’ has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2007) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters).

#### 3.5.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

#### 3.5.2. Exposure scenario/exposure model

Due to the scarce amount of data on QAs available in the EFSA Database and in the literature, the final data set on occurrence of QAs in food and feed was composed of data from very few samples. The QA profile and content in seeds can largely differ between species, cultivar, crop management and climate conditions. The occurrence data were received from 2 data providers and the representativeness of the data for the whole of Europe is uncertain.

Assuming a reduction of QAs by 89% as a result of debittering by water treatment and boiling represents a source of uncertainty because the obtained reduction is variable depending on how the process is carried out. It should be noted that no standardised method for debittering is available and the deviation from the described protocols may result in lower reductions.

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38 It should be noted that some authors recommend higher inclusion rates than used in this Opinion.
Limited consumption data on lupin seeds were available. Only for adults from one country a sufficient number of consumption days was available to calculate acute dietary exposure. On the contrary, no sufficiently reliable consumption data of lupin seeds were available for the other age groups such as children. Therefore, the exposure and consequently the risk could not be assessed for these groups under scenarios 1, 2 and 3.

When considering scenario 4, no data on consumption of lupin-based meat imitates were present in the EFSA Comprehensive Database; therefore, consumption of “meat imitates” was considered as a proxy of those and this may represent a source of overestimation.

For the exposure of farm animals, horses and companion animals, estimates were based on maximum recommended levels of inclusion of lupin seed meals in feed rations. In practice, however, inclusion rates will vary considerably, being influenced principally by the cost and availability of the lupin seeds relative to other feeds. This and the considerable variability that exists between feeding systems used for farm animals in Europe add to the uncertainty of animal exposure.

Levels of QAs in lupin seeds used to estimate exposure were derived from whole seeds, but both whole and dehulled seeds are used for food and feed. It has been assumed that levels and proportions of QAs in the seed coat and the kernel are similar, but if this is not the case then this assumption could lead to over- or under-estimation of exposure.

3.5.3. Model input (parameters)

Currently, no official standard methods are available for the analysis of QAs in foods. Analytical results used for the exposure assessment were therefore obtained using different analytical methods and varying LOQ/LODs. The difference between LB and UB exposure estimates for TotQA was limited, indicating that the uncertainty due to left-censored data is limited.

3.5.4. Other uncertainties

Studies in experimental animals

The acute toxicity of purified QAs has been investigated in experimental animals. Acute toxicity studies indicate that rats are less susceptible than mice or guinea pigs. All repeated-dose toxicity data are confined to rats. Repeated-dose studies have been performed using extracts from different Lupinus species, often containing different combinations of various QAs. Additional uncertainties in the interpretation of these studies arise from differences in the sources of QAs (flour versus seed extracts), cultivars, presence of lupin alkaloids other than QAs relevant for this opinion and the control diets used for the comparison.

Genotoxicity assays were performed with purified lupanine and sparteine as well as with L. termis extracts. While negative results were obtained for lupanine in a battery of assays, the data on sparteine are too limited to conclude regarding genotoxicity.

Observations in humans and hazard characterisation related to human health

Information on acute toxicity in humans is uncertain, as only sparse data were available from reports of poisoning cases with lupin seeds. The outcomes vary from fatal to less severe symptoms. Available reports do not allow to conclude on differences in toxicity of individual QAs or specific QA mixtures involved and on differences in the toxicity of seeds from different lupin species.

The reference point of 0.16 mg QA/kg bw identified by the Panel is derived by considering 20 mg of sparteine sulfate as the lowest single oral dose associated with pharmacological activity with respect to the endpoint of antiarrhythmic action. Uncertainty in the value of this reference point includes that information is missing (i) no information regarding the basis for this value, which was taken from old pharmacological reference literature, is available (ii) to which extent adverse effects may occur below the lowest-observed-effect-level (LOEL), (iii) where to allocate a NOEL which is covering all known pharmacological activities including the effects on the electrical conductivity in the heart and also the oxytocic (uterotonic) action on the pregnant uterus. Furthermore, differences in the individual sensitivity to sparteine due to CYP2D6 genetic polymorphism are difficult to be quantified and it is not known whether this polymorphism may affect the sensitivity to QAs other than sparteine.

In the hazard characterisation of the QAs, the principle of dose addition was used and a similar potency was assigned to all QAs relevant for this opinion which is a conservative approach considering that sparteine is the most potent QA in terms of toxicity. As the database on relative potencies is limited and there is no information on combined effects of exposure to multiple QAs vs exposure to...
single QAs, there is uncertainty in using dose-additivity with similar potencies in the hazard characterisation. Whereas it is acknowledged that interaction among QAs might occur both with regard to toxicokinetics and -dynamics, the CONTAM Panel considers it likely that grouping the QAs would result in an overestimation rather than an underestimation of the combined QA hazard.

**Studies in farm animals, horses and companion animals and hazard characterisation related to animal health**

Only sparse data are available on the kinetics of lupin QAs in farm animals, horses and companion animals. The information is limited to ruminants and pigs and no relevant studies have been performed with pure alkaloids.

Except for poultry and pigs, there is no information concerning the acute toxicity of QAs in livestock species. For repeated-dose exposure, no relevant toxicity studies dealing with pure QAs could be identified, except for salmonids. In the large majority of toxicity studies, only endpoints related to zootechnical performances have been taken into consideration. Most studies used lupin seeds as source of QAs in the diet and do not report the QAs present in the diet or their relative contribution to the total QA concentration. Such studies do not allow identification of NOAEL/LOAELs and therefore hazard characterisation was not possible for horses, companion animals and farm animals, with the exception of salmonids. Instead, as an alternative approach, the CONTAM Panel identified doses of QAs present in seeds from *L. luteus*, *L. albus* and *L. angustifolius* that can be tolerated with respect to zootechnical performance. Because of the wide species- and cultivar-related variation in total and specific QA content of lupin seeds, the calculated tolerated doses could not be extrapolated to all lupin species and cultivars.

For salmonids, similar NOAEL values were identified for lupinine and sparteine. In the absence of data on other QAs, the CONTAM Panel used this NOAEL for a group approach to characterise the hazard of the QAs relevant for this opinion and applied to all farmed salmonids. As for humans, the CONTAM Panel considers it likely that this grouping approach would result in an overestimation rather than an underestimation of the combined QA hazard.

3.5.5. Summary of uncertainties

In Table 15, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

**Table 15:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of quinolizidine alkaloids in lupin seeds and lupin-derived products.

| Sources of uncertainty                                                                 | Direction(a) |
|----------------------------------------------------------------------------------------|--------------|
| Very low number of food and feed samples available for exposure assessment              | +/-          |
| Extrapolation of occurrence data from 2 data providers to the whole of Europe           | +/-          |
| Application of a reduction factor of 89% for debittering                                | +/-          |
| Limited consumption data of lupin seeds from one country and only for adults            | +/-          |
| Lack of consumption data on lupin-based foods                                           | +/-          |
| Lack of information regarding QA levels in the husk and the proportion of the husk to the kernel | +/-          |
| Limited data on lupin seed consumption by farm and companion animals and horses in Europe | +/-          |
| Uncertainty in the reference point: pharmacologically active dose level                 | +/-          |
| Uncertainty in the reference point: use of sparteine as a representative for other QAs  | +/-          |
| Uncertainty regarding the application of NOAEL/LOAEELs for rainbow trout based on pure compounds to salmonids exposed to QAs from lupin seeds | +/—          |

QA: quinolizidine alkaloid; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; — = uncertainty with potential to cause under-estimation of exposure/risk.

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of QAs in lupin seeds and lupin-derived products is substantial and due to the limited data on occurrence and consumption, the risk characterisation is incomplete. Because the reference point for QAs used for human risk assessment is based on the effects for sparteine, which is assumed to be the most potent QA, the CONTAM Panel concluded that the risk is more likely to be overestimated than...
underestimated. Risk characterisation was not possible for farmed animals other than salmonids nor it was possible for horses and companion animals.

4. Conclusions

4.1. Hazard identification and characterisation

4.1.1. Toxicokinetics

- In humans and experimental animals, sparteine and lupanine are rapidly absorbed, widely distributed and rapidly eliminated in urine mainly unchanged and as oxidised metabolites. Sparteine CYP2D6-mediated oxidation in humans may be affected by defective CYP2D6 activity occurring in about 5–10% of Caucasians due to genetic polymorphisms resulting in slow sparteine elimination.
- In ruminants, there is extensive but slow absorption and slow elimination of lupanine and 5,6-dehydrolupanine. In pigs, lupanine seems to be extensively absorbed and excreted in urine mainly unmodified together with the metabolites isolupanine and OH-lupanine.
- There is no evidence of the formation of conjugated metabolites of sparteine and lupanine in mammalian species.
- No information is available to assess transfer rates of QAs in food of animal origin; however, based on indirect evidence possible transfer to milk should be considered.

4.1.2. Toxicity in laboratory animals

- Sparteine is approximately two- to three-fold more acutely toxic than lupanine both in mice and guinea pigs. Mice appear to be more sensitive than rats to acute toxicity of lupanine. In rats, the acute toxicity of lupanine and 13α-OH-lupanine is similar.
- Acute symptoms following oral administration of lupanine, 13α-OH-lupanine and sparteine are similar in rats, mice and guinea pigs, with death resulting from respiratory failure and toxic effects affecting the CNS.
- The identified repeated-dose toxicity studies could not be used for the evaluation of QA toxicity.
- Several QAs were unable to bind or intercalate into DNA. Lupanine and extracts of *L. termis* (main QA being lupanine) did not induce gene mutations in bacteria and in mammalian cells *in vitro* or micronuclei *in vivo*. The data on sparteine are too limited to conclude regarding genotoxicity.

4.1.3. Observations in humans

- Intoxication with lupin seeds do not occur frequently and rarely lead to a fatal outcome. Reports suggest that children are more susceptible to intoxication with lupin alkaloids than adults and that doses from 10 mg/kg bw onwards may be lethal for children. Typical symptoms refer to the anticholinergic syndrome, are of neurological nature and may also affect the digestive and/or cardiovascular systems.
- Intoxication have been associated with inadequate debittering of lupin seeds by consumers. No case of poisoning has been identified which was attributed to consumption of industrially produced lupin seed-based food.
- Sparteine was used therapeutically in the past for both its antiarrhythmic and oxytocic properties in humans.
- In the treatment of cardiac arrhythmia, the recommended daily dosage for (-)-sparteine sulfate ranged from 800 to 1,000 mg per day (equivalent to 6.4–8 mg sparteine/kg bw for a 70-kg individual) for short-term use and from 400 to 500 mg/day (3.2–4 mg sparteine/kg bw) for long-term therapy. Adverse effects of anticholinergic nature were reported at these therapeutic doses. The lowest oral dose reported with antiarrhythmic effects was 20 mg (equivalent to 0.16 mg sparteine/kg bw).
- Sparteine sulfate may cause uterotonic (oxytocic) effects in pregnant women.
- Sparteine blocks the transmission of signals from the nerves to the muscles like curare, which may lead to respiratory arrest and death. From a poisoning case, it is known that a dose of 30 mg of sparteine/kg bw was fatal to a young child.
No adverse effects were observed after administration of a single oral dose of 10 mg of lupanine or 10 mg 13α-OH-lupanine (each equivalent to 0.14 mg/kg bw; body weight of 70 kg) to volunteers who were EMs or PMs.

4.1.4. Farm animals, horses and companion animals

- Field cases of pig poisoning by QAs are characterised by depression of feed intake and reduced weight gain; severely affected animals show vomiting, recumbency, abortion and deaths characterised by gastric torsion and gastro-intestinal bloat.
- LD50 values for sparteine and lupanine in poults confirm the higher (two- to three-fold) toxicity of the former observed in guinea pigs and mice.
- Most tolerance studies in food producing species were not performed with pure QAs and were based only on lupin impact on zootechnical performances (feed intake, weight gain, milk and egg production).
- For cattle, 50 mg/kg bw per day was identified as a tolerated dose for the sum of lupanine and 13α-OH-lupanine present in seeds from *L. albus*. No relevant studies were identified for sheep and goats.
- For pigs, 1–10 and 1.5 mg/kg bw per day were identified as tolerated doses of lupin alkaloids present in seeds from *L. angustifolius* and *L. luteus*, respectively.
- Based on zootechnical performance, 1 mg/kg bw per day was identified as a dose of lupin alkaloids present in seeds from *L. albus* that can be tolerated by chickens. In one-day-old quail chicks, 826 mg/kg bw was identified as a dose of lupin alkaloids present in seeds from *L. albus* that can be tolerated (only dose tested).
- For rabbits, 30 mg/kg bw per day was identified as a tolerated dose of lupin alkaloids present in seeds from *L. albus*.
- For rainbow trout a NOAEL of 1 mg/kg bw per day for lupinine and sparteine could be calculated based on the reduction of feed intake and weight gain and the increase in hepatosomatic index. The LOAEL was 2.5 mg/kg bw per day for lupinine and 3.5 mg/kg bw per day for sparteine.
- No reliable data were identified for horses, cats or dogs.

4.1.5. Mode of action

- The most important mode of action of QAs in relation to acute toxicity is their binding to and inhibitory action on AChRs producing an anticholinergic syndrome which may include paralysis of respiratory muscles causing respiratory failure and death.
- The different QAs bind with different affinity to the nAChRs or mAChRs.
- QAs may also cause, with different potencies, inhibition of voltage dependent ion channels, e.g. sodium and potassium channels, and calcium activated potassium channels, effects possibly underlying their antiarrhythmic effect.
- The uterotonic effects of sparteine appear to be mediated via an increased production of prostaglandin F.
- For QAs relevant in this opinion, affinities to AChRs appear to be in the same range or lower than those of sparteine. Scarce data on other modes of action of some QAs indicate that these QAs are similarly or less active than sparteine.

4.1.6. Possibilities for derivation of a HBGV

- Anticholinergic effects and effects on electric conductivity in the heart following acute exposure to sparteine are considered to be the critical effects for human hazard characterisation. The lowest described single oral antiarrhythmic dose of 0.16 mg sparteine/kg bw was selected as a reference point to characterise the risk following acute exposure.
- Because of similar modes of action for QAs, the CONTAM Panel considers it appropriate to use a group approach and apply the principle of dose-additivity with equal potencies of the QAs for their combined effect.
- Overall, the available data on dose-effect relationships were considered insufficient to propose an HBGV and instead an MOE approach was used.
- No reference point could be identified to characterise the risk following chronic exposure.
4.2. Occurrence and exposure

4.2.1. Food

- The highest mean concentrations of TotQA were found in ‘lupins (dry) and similar’ (429 mg/kg) and lupin-based coffee imitate (powder) (331 mg/kg). High concentrations of QAs in lupin-based coffee imitates have also been reported in scientific literature.
- Lupanine was the most abundant QA in seeds from \textit{L. albus} and \textit{L. angustifolius}, followed by 13\alpha-\text{OH}-lupanine (especially abundant in \textit{L. angustifolius}) and angustifoline.
- Between 89% and 97% of the QAs present in seeds are removed by water treatment and boiling. Studies of the influence of germination on the QA concentrations show no clear effect.
- Due to the limited occurrence and consumption data, the CONTAM Panel decided to calculate acute dietary exposure only for a few food groups under specific scenarios.
- Three scenarios on dietary exposure of adults from lupin seeds were elaborated. The highest exposure to TotQA from lupin seeds was calculated for a scenario in which no debittering of the dry lupin seeds was performed, resulting in exposures up to 1,700 μg/kg bw per day. Assuming a debittering step, resulting in a decrease of the TotQA by 89%, the P95 dietary exposure ranges between 84 (based on average QA content) and 187 μg/kg bw per day (based on high QA content). For a scenario based on ready-to-eat lupin seeds, the P95 exposure was 44–46 μg/kg bw per day.
- A scenario on lupin-based meat imitates showed P95 dietary exposures up to 194–228 μg/kg bw per day (LB–UB).

4.2.2. Feed

- Analytical results from 11 samples of lupin seeds from \textit{L. angustifolius} used as feed were available with a mean TotQA concentration of 257 mg/kg. Due to the limited number of feed samples available for the dietary exposure assessment, the data set was supplemented with food samples.
- Lupanine and 13\alpha-\text{OH}-lupanine accounted each for about 36% of the TotQA content in these samples.
- Exposures to QAs by farm animals, horses and companion animals were estimated using maximum recommended inclusion rates of lupin seeds in livestock diets and therefore are likely to represent worst-case scenarios. In addition, insufficient data on levels of QAs in lupin seeds were available to allow P95 estimates of exposure to be made.
- In the category ‘Ruminants and horses’, the highest exposure was for lactating goats (3.3 mg/kg bw per day). For pigs and poultry, the highest exposure was for fattening chickens and laying hens (3.5 mg/kg bw per day). For salmonids, the exposure was 1.6 mg/kg bw per day and for rabbits 4.4 mg/kg bw per day. For cats and dogs, the exposure was 0.6 mg/kg bw per day.

4.3. Risk characterisation

- This risk assessment relates to lupin seeds and lupin-derived products with a QA profile comparable with that of the submitted data and should not be extrapolated to lupin seeds with a different QA profile (e.g. lupin seeds from species and/or varieties high in sparteine).

4.3.1. Human health

- Due to the limited occurrence and consumption data, no full risk characterisation was possible. The risk from certain lupin-based foods such as pasta, bread and coffee imitates could not be assessed, nor the risk to children from lupin seed consumption.
- There is uncertainty linked to the reference point of 160 μg sparteine/kg bw. This reference point for QAs is based on the effects of sparteine which is the most potent QA with respect to toxicity. However, sparteine occurs only in trace amounts according to the data submitted to EFSA and, according to clinical experience, doses 7.5-10 times higher than the reference point ensure a reliable antiarrhythmic effect. Therefore, the CONTAM Panel concluded that MOEs > 1 do not indicate a health concern.
• In the event that lupin seeds were not debittered and for a scenario reflecting dietary exposure from lupin-based meat imitates, the CONTAM Panel calculated MOE values in the range of 0.1–0.8. These MOEs may indicate a concern.
• In a scenario applying a reduction factor for debittering, the calculated MOEs are of lower concern (0.9; high QA concentration) or no concern (1.9; average QA concentration).
• MOEs ≥ 1 were calculated for ready-to-eat lupin seeds that had an average QA content.

4.3.2. Animal health

• For salmonids, the CONTAM Panel applied a group approach and used a NOAEL of 1 mg/kg bw per day for the relevant QAs in this opinion. The mean dietary exposure of salmonids is above the NOAEL but below the LOAEL of 2.5 mg/kg bw per day. The CONTAM Panel considers the risk for adverse effects in salmonids to be low.
• For the other species, the available database on adverse effects was too limited to identify overall NOAEL/LOAELs. Instead, as an alternative approach, the CONTAM Panel identified doses of QAs present in lupin seeds that can be tolerated with respect to zootechnical performance. However, such a dose is only applicable to lupin seeds with similar QA composition as used in the study from which the dose was derived and does not allow a full characterisation of the risk.
• For cattle and rabbits, the mean dietary exposure is well below the tolerated dose.
• For pigs and chickens, the mean dietary exposure is in the same range as the dose that can be tolerated.

5. Recommendations

• Further studies on ADME of QAs in humans and animal species are needed.
• There is a need for repeated-dose toxicity studies on the QAs considered in this opinion. This also includes studies on relative potencies and combined actions of QAs as they occur in mixtures in food and feed.
• There is a need for more data on the transfer of QAs from feed to food of animal origin.
• More information on consumption of lupin seeds and lupin-based food in the EU is needed.
• Reference standards for QAs other than lupanine, lupinine and sparteine should become available, as well as certified reference materials and international proficiency tests.

Documentation provided to EFSA

Data on the alkaloid content of lupin seeds. July 2019. Submitted by Inveja during the public consultation. (see Section 2.1).

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**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| A            | Australian |
| ACh          | acetylcholine |
| AChE         | acetylcholinesterase |
| AChR         | acetylcholine receptor |
| ADME         | absorption, distribution, metabolism, and excretion |
| AE           | Australian extruded |
| ALT          | alanine transaminase |
| ANZFA        | Australia New Zealand Food Authority |
| AST          | aspartate transaminase |
| BchE         | butyrylcholinesterase |
| BFR          | Bundesinstitut für Risikobewertung/German Federal Institute for Risk Assessment |
| BL           | bitter lupin |
| bw           | body weight |
| Cmax         | maximum plasma concentrations |
| CAS          | Chemical Abstracts Service |
| CNS          | central nervous system |
| CONTAM Panel | The EFSA Panel on Contaminants in the Food Chain |
| COT          | Committee on Toxicity |
| CYP          | cytochrome P450 |
| DCL          | detoxified crushed lupin seeds |
| DIC          | instant controlled pressure drop technology |
| DM           | dry matter |
| dw           | dry weight |
| DWL          | ground dehulled |
| EC50         | half maximal effective concentration |
| EEC          | European Economic Community |
| Acronym | Description |
|---------|-------------|
| OECD    | Organisation for Economic Co-operation and Development |
| PM      | poor metabolisers |
| PO      | per os (by mouth) |
| P95     | 95th percentile |
| PTDI    | provisional tolerable daily intake |
| QA      | quinolizidine alkaloid |
| RBL     | raw blue lupin |
| RYL     | raw yellow lupin |
| SGOT    | serum glutamic-oxaloacetic transaminase |
| SGPT    | serum glutamic-pyruvate transaminase |
| SL      | sweet lupin |
| SMBL    | soaked micronised bitter lupin |
| SSD     | standard samples description |
| SSB     | Single-strand break |
| TD      | coefficient of crude protein digestibility |
| TK      | toxicokinetic |
| TG      | triacylglycerol |
| TLC     | thin-layer chromatography |
| Tmax    | maximum plasma concentration |
| TotQAs  | Total QAs |
| TTM     | Traditional Turkish method |
| UB      | upper bound |
| UF      | uncertainty factor |
| UV      | ultraviolet |
| Vmax    | maximum velocity |
| WHO     | World Health Organization |
| WL      | ground whole |
| WW      | wet weight |
Appendix A – Major quinolizidine alkaloids present in *Lupinus* and related genera from the Genisteae tribe

| Compound                  | Chemical structure | CAS No: | MW:       | LogP (est): |
|---------------------------|--------------------|---------|-----------|-------------|
| (-)-Lupinine              | ![Image](image1.png) | 486-70-4| 169.268   | 1.2         |
| (+)-Epilupinine           | ![Image](image2.png) | 486-71-5| 169.268   | 1.1         |
| (-)-Cytisine              | ![Image](image3.png) | 485-35-8| 190.246   | 0.2         |
| (-)-N-Methylcytisine      | ![Image](image4.png) | 486-86-2| 204.273   | 1.3         |
| (-)-Albine                | ![Image](image5.png) | 53915-26-7| 232.327 | 1.3         |
| 2,3-Dehydrosparteine      | ![Image](image6.png) | 67528-17-0| 232.364 | 2.7         |
| 5,6-Dehydrosparteine      | ![Image](image7.png) | 2130-67-8| 232.364 | 2.5         |
| Compound                      | Chemical structure |
|-------------------------------|--------------------|
| (-)-Angustifoline             | ![Chemical structure](image1) |
| Synonym: Jamaicensine         | CAS No: 550-43-6   |
| C_{14}H_{22}N_{2}O            | MW: 234.343        |
| LogP (est): 1.4               |                    |
| Isoangustifoline              | ![Chemical structure](image2) |
| CAS No: 82189-28-4            |                    |
| C_{14}H_{22}N_{2}O            | MW: 234.343        |
| LogP (est): 1.4               |                    |
| (-)-Sparteine                 | ![Chemical structure](image3) |
| Synonym: Lupinidine           | CAS No: 90-39-1    |
| C_{15}H_{26}N_{2}             | MW: 234.387        |
| LogP (est): 2.5               |                    |
| (-)-α-Isosparteine            | ![Chemical structure](image4) |
| Synonym: Genisteine alkaloid  | CAS No: 446-95-7   |
| C_{15}H_{26}N_{2}             | MW: 234.387        |
| LogP (est): 2.5               |                    |
| (-)-Sparteine                 | ![Chemical structure](image5) |
| Synonym: Pachycarpine         | CAS No: 492-08-0   |
| C_{15}H_{26}N_{2}             | MW: 234.387        |
| LogP (est): 2.5               |                    |
| (-)-Anagyrine                 | ![Chemical structure](image6) |
| Synonym: Monolupine, rhombinine| CAS No: 486-89-5   |
| C_{15}H_{26}N_{2}O            | MW: 244.338        |
| LogP (est): 1.6               |                    |
| (-)-Multi florine             | ![Chemical structure](image7) |
| Synonym: N-Methylalbine       | CAS No: 529-80-6   |
| C_{15}H_{22}N_{2}O            | MW: 246.354        |
| LogP (est): 1.5               |                    |
| 11,12-seco-12,13-Didehydromultiflorine | Synonym: N-Methylalbine |
| C_{15}H_{22}N_{2}O            | MW: 246.354        |
| LogP (est): 1.7               |                    |
| Compound | Chemical structure |
|----------|--------------------|
| 5,6-Didehydrolupanine |
| CAS No: 32101-29-4 |
| \(\text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}\) |
| MW: 246.354 |
| LogP (est): 1.3 |
| ![Chemical structure of 5,6-Didehydrolupanine](image1) |
| Tetrahydorhombifoline |
| CAS No: 3382-84-1 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}\) |
| MW: 248.370 |
| LogP (est): 1.8 |
| ![Chemical structure of Tetrahydorhombifoline](image2) |
| (+)-Lupanine |
| Synonym: \(\alpha\)-Lupanine, 2-oxosparteine |
| CAS No: 550-90-3 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}\) |
| MW: 248.370 |
| LogP (est): 1.6 |
| ![Chemical structure of (+)-Lupanine](image3) |
| (+)-\(\alpha\)-Isolupanine |
| Synonym: 11-Isolupanine |
| CAS No: 486-87-3 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}\) |
| MW: 248.370 |
| LogP (est): 1.6 |
| ![Chemical structure of (+)-\(\alpha\)-Isolupanine](image4) |
| (-)-17-Oxosparteine |
| CAS No: 489-72-5 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}\) |
| MW: 248.370 |
| LogP (est): 1.7 |
| ![Chemical structure of (-)-17-Oxosparteine](image5) |
| 3\(\beta\)-OH-lupanine |
| Synonym: 4-OH-lupanine |
| CAS No: 129443-39-6 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}_{2}\) |
| MW: 264.369 |
| LogP (est): 0.6 |
| ![Chemical structure of 3\(\beta\)-OH-lupanine](image6) |
| (+)-13\(\beta\)-OH-lupanine |
| Synonym: Jamaidine, luparine |
| CAS No: 15358-48-2 |
| \(\text{C}_{15}\text{H}_{24}\text{N}_{2}\text{O}_{2}\) |
| MW: 264.369 |
| LogP (est): 0.6 |
| ![Chemical structure of (+)-13\(\beta\)-OH-lupanine](image7) |
| Compound                              | Chemical structure | CAS: Chemical Abstracts Service; MW: molecular weight. |
|--------------------------------------|--------------------|------------------------------------------------------|
| 3β,13α-diol-lupanine                 | ![Chemical structure](image1) | CAS No: 101512-24-7 C_{15}H_{24}N_{2}O_{3} MW: 280.368 LogP (est): -0.4 |
| 13α-Angeloyloxylupanine              | ![Chemical structure](image2) | CAS No: 72822-06-1 C_{20}H_{30}N_{2}O_{3} MW: 346.471 LogP (est): 2.4 |
| 13α-Tigloyloxylupanine               | ![Chemical structure](image3) | CAS No: 57943-34-7 C_{20}H_{30}N_{2}O_{3} MW: 346.471 LogP (est): 2.4 |
| 13α-cis-cinnamoyloxylupanine         | ![Chemical structure](image4) | CAS No: 86707-49-5 C_{24}H_{30}N_{2}O_{3} MW: 394.515 LogP (est): 3.3 |
| 13α-trans-cinnamoyloxylupanine       | ![Chemical structure](image5) | CAS No: 5835-04-1 C_{24}H_{30}N_{2}O_{3} MW: 394.515 LogP (est): 3.3 |
Appendix B – Other relevant alkaloids present in *Lupinus* and related genera from the Genisteae tribe

| Compound      | Chemical structure |
|---------------|--------------------|
| Gramine       | ![Gramine chemical structure](image) |
| Synonym: 3-(Dimethylaminomethyl)indole |                     |
| CAS No: 87-52-5 |                     |
| C<sub>11</sub>H<sub>14</sub>N<sub>2</sub> |                     |
| MW: 174.247   |                     |
| LogP (est): 1.8 |                    |

| Ammodendrine  | ![Ammodendrine chemical structure](image) |
|---------------|------------------------------------------|
| Synonym: Spherocarpine |                        |
| CAS No: 494-15-5 |                        |
| C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>O |                        |
| MW: 208.305  |                        |
| LogP (est): 0.3 |                        |

CAS: Chemical Abstracts Service; MW: molecular weight.
Appendix C – Biosynthetic pathway of ester-type quinolizidine alkaloids in *Lupinus* species (Bunsupa et al., 2013)\textsuperscript{39}

LDC: lysine decarboxylase; HMT/HLT: tigloyl-CoA: (-)-13α-hydroxymultiflorine/(+)-13α-hydroxylupanine O-tigloyltransferase; ECT/EFT-LCT/LFT, p-coumaroyl-CoA/feruloyl-CoA: (+)-epilupinine/(-)-lupinine O-coumaroyl/feruloyltransferase.

\textsuperscript{39} Reprinted by permission from Springer: Springer Berlin Heidelberg, Biotechnology for Medicinal Plants: Micropropagation and Improvement, (Eds Chandra S, Lata H and Varma A), Bunsupa S, Saito K and Yamazaki M. Molecular Biology and Biotechnology of Quinolizidine Alkaloid Biosynthesis in Leguminosae Plants, 263–273, 2013.
Appendix D – Occurrence of QAs in lupin seeds

Tables D.1–D.4 give an overview of concentrations of QAs in seeds of the species *L. albus*, *L. luteus*, *L. angustifolius* and *L. mutabilis*. Papers in which the analytical method is not described, or when titration or TLC was used for determination, are not included. The columns ‘QA composition’ and ‘concentration ranges of QAs’ show the concentration of the 11 QAs that are considered as relevant for this opinion while the occurrence of other QAs as well as other alkaloids is shown in ‘Comments’. The relevant QAs are sorted according to concentration so the QA with the highest concentration is mentioned first.

In many papers, the ‘total alkaloid’ concentration is reported. However, it is not always clear whether this refers to the total of QAs or the total of all alkaloids. It should be noted that some of the applied analytical methods are not specific for QAs so it cannot be concluded that ‘total’ only included QAs. In some papers, it is clearly stated that ‘total’ means the sum of the found QAs and this is indicated in the tables. When the authors have indicated if the seeds can be considered as bitter or sweet, this is also noted in the table. Where the stereochemistry was not provided in the paper, the CONTAM Panel assumed that the isomer present is the naturally occurring form in that lupin species, e.g. 13α-OH-lupanine in case of 13-OH-lupanine and α-isolupanine in case of isolupanine.

It has been tried to give all results in percentage of the total even though other units have been used in the papers. If this was not possible, the unit is stated in the tables. Due to rounding, the sum of the percentages for the individual QAs is not necessarily 100%. Some of the concentrations are given on a dry matter basis, but due to high dry matter content of the seeds, concentrations given on wet weight and dry matter are not very different.

Table D.5 summarises the outcome of different reviews regarding the occurrence of QAs in the four relevant lupin species and Table D.6 summarises the results on QA occurrence in lupin leaves.
# Table D.1: Occurrence of QAs in seeds of *L. albus*

| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%)<sup>(e)</sup> | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|------------------------|---------------------------------|----------------|---------------------------------------------|----------------------------------------|--------------------------------------|-----------|
| Multitalia, P2, P3, 4-3, 7-44, 7-7, 8-28, 8-483 | 47–6,392 mg/kg<sup>(g)</sup> | Lupanine, Multiflorine, Albine, 13α-OH-lupanine, 13α-Angeloyloxylupanine, Angustifoline, α-Isolupanine, 13α-Tigloyloxylupanine | 5,395 mg/kg Not detected-616 mg/kg 10–167 mg/kg Not-detected-126 mg/kg Not detected-112 mg/kg Not detected-84 mg/kg Not detected-75 mg/kg Not detected-38 mg/kg | GC-MS | All varieties grown at two different places in Italy in two growing seasons | Annichiarico et al. (2014) |
| 48 types, rich in alkaloids (world-wide) | < 1,000–about 16,000 mg/kg<sup>(g),(c)</sup> | Lupanine, Other QAs: albine > 13α-OH-lupanine > angustifoline > 13α-angeloyloxylupanine | 88–92 | GC-MS | | Boschin et al. (2008) |
| Alkaloid-poor: Adam, Ares, Energy, Lucky, Ludic, Lustar | 50–565 mg/kg<sup>(f)</sup> | Lupanine, 13α-Angeloyloxylupanine, 13α-OH-Lupanine, Albine, 13α-Tigloyloxylupanine, Angustifoline, α-Isolupanine | 26–256 mg/kg Not detected-88 mg/kg 7–79 mg/kg 10–68 mg/kg Not detected-28 mg/kg Not detected-22 mg/kg | GC-MS | Dehulled seeds | Boschin et al. (2008) |
| LO-3923, bitter (N = 8) | 23,600 mg/kg | Lupanine, Albine, Multiflorine, Angustifoline, 13α-OH-Lupanine, α-Isolupanine, 13α-Tigloyloxylupanine | 58 19 7.6 1.1 0.68 0.30 0.08 | GC-NPD and GC-MS | From Spain Ammodendrine, 5,6-Dehydrolupanine, 11,12-seco-12,13-didehydromultiflorine | de Cortes Sánchez et al. (2005) |
| Bitter | 14,400 mg/kg DM<sup>(e)</sup> | Lupanine, Multiflorine, α-Isolupanine, Albine, Sparteine | 87 11 1.0 0.63 0.13 | GC-FID | | Erbas (2010) |
| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%)<sup>(e)</sup> | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|------------------------|--------------------------------|----------------|-----------------------------------------------|---------------------------------------|----------------------------------------|-----------|
| Luxor, Rosetta         | 38–75 mg/kg<sup>(a)</sup>     | Lupanine 13α-OH-Lupanine Angustifoline α-Isolupanine | 18–33 mg/kg 3.8–13.4 mg/kg 4.9–6.3 mg/kg 1.8–2.5 | GC-MS LOQ = 0.2–0.4 mg/kg | Grown in Italy 11,12-Dehydrolupanine | Gresta et al. (2010) |
| Multitalia             | 1,664<sup>(a)</sup> mg/kg     | Lupanine 13α-OH-Lupanine Angustifoline α-Isolupanine Sparteine | 1,499 mg/kg 99 mg/kg 34 mg/kg 9.1 mg/kg 2.1 mg/kg | GC-MS LOQ = 0.2–0.4 mg/kg | Grown in Italy 11,12-Dehydrolupanine | Gresta et al. (2010) |
| Present and old cultivars, 59 in total | 200–49,100 mg/kg | Lupanine 13α-OH-Lupanine Multiflorine Albine Angustifoline | 28–72 2.4–33 1.0–22 0.02–16 0.29–10 | GC<sup>(d)</sup> | Seeds from Poland 11,12-seco-12,13-Didehydromultiflorine | Kroc et al. (2017) |
| Amiga, Lumen, Estoril, 2 × Multitalia | 220–52,380 mg/kg<sup>(b)</sup> | Lupanine 13α-OH-Lupanine Angustifoline α-Isolupanine Lupinine | 160–46,240 mg/kg 0–4,710 mg/kg 10–750 mg/kg 10–250 mg/kg Not quantified-50 mg/kg | GC-FID and GC-IT-MS | Grown in France, Portugal and Italy 11,12-Dehydrolupanine | Magalhães et al. (2017) |
| Bitter and sweet       | 143–226 mg/kg<sup>(b)</sup> | 13α-OH-Lupanine Lupanine Angustifoline Albine α-Isolupanine 13α-Angeloyloxylupanine 13α-Tigloyloxylupanine | 48–117 mg/kg 69–111 mg/kg 9–12 mg/kg Traces Traces Traces | GC-FID and GC-MS | Ammodendrine Dihydroxylupanine | Reinhard et al. (2006) |
| Ares                   | 146 mg/kg<sup>(a)</sup>       | Lupanine 13α-Angeloyloxylupanine 13α-OH-Lupanine Albine 13α-Tigloyloxylupanine | 43 23 14 11 8.9 | GC-MS | | Resta et al. (2008) |
| Tytop                  | 1,247 mg/kg                   | Lupanine 13α-OH-Lupanine Albine 13α-Angeloyloxylupanine Multiflorine | 77 11 6.0 3.7 2.6 | GC-MS | | Resta et al. (2008) |
| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%)\(^{(e)}\) | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|------------------------|---------------------------------|----------------|----------------------------------------|----------------------------------------|--------------------------------------|-----------|
| Aster, Lutteur, Luxor, Rosetta, Multitalia 1 (sweet) | 67–1,656 mg/kg\(^{(b)}\) | Lupanine\(^{(h)}\) Albine 13α-OH-Lupanine Angustifoline 13α-Tigloyloxy-lupanine Tetrahydro-rhombifoline | 30–1,379 mg/kg 12–162 mg/kg 3–157 mg/kg 3.6–65 mg/kg 6.4–44 mg/kg 1.0–13 mg/kg | GC–MS | Grown in Italy 3ji-Tigloyloxy-lupanine | Romeo et al. (2018) |
| Lublanc\(^{(g)}\) | 14,516 mg/kg\(^{(b)}\) | Lupanine\(^{(h)}\) Albine 13α-OH-Lupanine Angustifoline 13α-Tigloyloxy-lupanine Tetrahydro-rhombifoline | 11,219 mg/kg 1,012 mg/kg 492 mg/kg 268 mg/kg 102 mg/kg 24 mg/kg | GC–MS | Grown in Italy 3ji-Tigloyloxy-lupanine | Romeo et al. (2018) |
| Multitalia 2,3 and 4, Puglia, Molise, Basilicata, Scili, Modica, Calabria 1, 2, 3, 4 | 3,185–19,340 mg/kg\(^{(b)}\) | Lupanine\(^{(h)}\) Albine 13α-OH-Lupanine Angustifoline 13α-Tigloyloxy-lupanine Tetrahydro-rhombifoline | 2,161–14,480 mg/kg 375–2,596 mg/kg 158–893 mg/kg 42–518 mg/kg 51–348 mg/kg 6.5–44 mg/kg | GC–MS | Grown in Italy 3ji-Tigloyloxy-lupanine | Romeo et al. (2018) |
| Not given | Not given | Lupanine Albine 13α-OH-Lupanine Multiflorine Sparicine Angustifoline α-Isolupanine 13α-Angeloyloxy-lupanine Unknown QAs | 70 15 8 3 < 1 < 1 < 1 < 1 | GC–MS | Analysis of 56 lupin species for 100 QAs | Wink et al. (1995) |

**DM:** dry matter; **FID:** flame ionisation detector; **GC–IT-MS:** gas chromatography–ion trap mass spectrometry; **GC–MS:** gas chromatography–mass spectrometry; **LOD:** limit of detection; **LOQ:** limit of quantification; **N:** number of samples; **NPD:** nitrogen-phosphorus detector; **QA:** quinolizidine alkaloids.

\(^{(a)}\): Sum of individual QAs.
\(^{(b)}\): Sum of alkaloids.
\(^{(c)}\): Total for all 48 types; numbers read from a figure with a broad scale.
\(^{(d)}\): Detector not reported.
\(^{(e)}\): Given as percentage of total QA, unless another unit is given. In the latter case, the percentage of the individual QAs was not reported by the authors.
\(^{(f)}\): Not all QAs found in all samples so the lowest total amount is not the sum of the lowest amounts of the individual QAs.
\(^{(g)}\): It is stated in the paper to be a sweet variety but with a total concentration of more than 10,000 m/kg it is not included as sweet in this opinion but as bitter.
\(^{(h)}\): Only results for QAs found in a sweet variety are shown in the paper.
Table D.2: Occurrence of QAs in seeds of *L. angustifolius*

| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%) | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|------------------------|---------------------------------|----------------|--------------------------------|----------------------------------------|--------------------------------------|-----------|
| Alkaloid poor: Arabella, Jindalee, Quilinoc | 141–701 mg/kg\(^{(a)}\) | Lupanine 13\(^{a}\)-OH-Lupanine Angustifoline \(\alpha\)-Isolupanine | 92–426 mg/kg 26–183 mg/kg 12–63 mg/kg 8–34 mg/kg | GC-MS | | Boschin et al. (2008) |
| Graf | 1,450 mg/kg\(^{(a)}\) | Lupanine 13\(^{a}\)-OH-Lupanine \(\alpha\)-Isolupanine Angustifoline | 69 24 5.8 0.66 | GC-NPD | From a Polish breeding station Ammodendrine 3\(j\)-OH-Lupanine | Chilomer et al. (2010) |
| W26, Polonez (sweet) | 840–1,800 mg/kg DM\(^{(a)}\) | Lupanine 13\(^{a}\)-OH-Lupanine \(\alpha\)-Isolupanine Angustifoline Sparteine Tetrahydrorhombifoline | 4.0–50 19–21 6.4–14 2.7–4.4 2.5–4.8 0.0–1.0 | GC-MS | Isoangustifoline | Christiansen et al. (1997) |
| Zubr (bitter) | 20,600 mg/kg DM\(^{(a)}\) | Lupanine 13\(^{a}\)-OH-Lupanine Angustifoline Tetrahydrorhombifoline \(\alpha\)-Isolupanine Sparteine | 71 17 5.8 2.8 1.2 0.1 | GC-MS | Isoangustifoline | Christiansen et al. (1997) |
| Not given (\(N = 8\)) | 14,740 mg/kg\(^{(a)}\) | Lupanine Angustifoline 13\(^{a}\)-OH-Lupanine Multiflorine | 36 30 25 0.95 | GC-NPD and GC-MS | From Spain Isoangustifoline, 5,6-Dehydrolupanine, 11,12-seco-12,13-Didehydromultiflorine Unidentified ester | de Cortes Sánchez et al. (2005) |
| Wonga, Jindale, Sonet | 15–55 mg/kg\(^{(a)}\) | 13\(^{a}\)-OH-Lupanine Lupanine Angustifoline Sparteine | 5.6–26 mg/kg 7.4–17.9 mg/kg < LOQ–11.2 mg/kg < LOQ–0.59 mg/kg | GC-MS LOQ = 0.2–0.4 mg/kg | Grown in Italy | Gresta et al. (2010) |
| Past and present cultivars, 78 in total | 652–19,176 mg/kg\(^{(a)}\) | Lupanine 13\(^{a}\)-OH-Lupanine Angustifoline \(\alpha\)-Isolupanine | 43–54 29–37 14–17 1.4–5.8 | GC\(^{(c)}\) | Seeds from Poland | Kamel et al. (2016) |
| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%)(d) | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|-----------------------|---------------------------------|----------------|-----------------------------------|----------------------------------------|--------------------------------------|-----------|
| Azuro, Sonet          | 690-24,950 mg/kg                | Lupanine Angustifoline α-Isolupanine Sparteine | 550-20,570 mg/kg 90-3,830 mg/kg 40-550 mg/kg Not detected-10 mg/kg | GC-FID and GC-IT-MS | Grown in Portugal and Poland 11,12-Dehydrolupanine | Magalhães et al. (2017) |
| Bitter (N = 3)        | 10,800-19,800 mg/kg             | Lupanine 13α-OH-Lupanine α-Isolupanine | 4,000-12,900 mg/kg 5,000-6,600 mg/kg 213-237 mg/g | GC-FID and GC-MS | | Reinhard et al. (2006) |
| Sweet (N = 8)         | 44-2,120 mg/kg                  | 13α-OH-Lupanine Lupanine Angustifoline α-Isolupanine | 18-943 mg/kg 18-862 mg/kg 7-226 mg/kg 37-90 mg/kg | GC-FID and GC-MS | α-Isolupanine only in samples from Australia | Reinhard et al. (2006) |
| Boregine              | 1,107 mg/kg                     | Lupanine Angustifoline 13α-OH-Lupanine 13α-Isolupanine | 50 | GC-MS | | Resta et al. (2008) |
| Not given             | Not given                       | Lupanine 13α-OH-Lupanine Angustifoline Sparteine Tetrahydrorhombifoline α-Isolupanine | 70 12 10 < 1 < 1 < 1 | GC-MS | Analysis of 56 lupin species for 100 QAs | Wink et al. (1995) |

DM: dry matter; FID: flame ionisation detector; GC-IT-MS: gas chromatography-ion trap mass spectrometry; GC-MS: gas chromatography–mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; N: number of samples; NPD: nitrogen-phosphorus detector; QA: quinolizidine alkaloids.

(a): Sum of individual QAs.
(b): Sum of alkaloids.
(c): Detector not reported.
(d): Given as percentage of total QA, unless another unit is given. In the latter case, the percentage of the individual QAs was not reported by the authors.
Table D.3: Occurrence of QAs in seeds of *L. luteus*

| Variety/cultivar/breed | Total QA concentration in seeds | QA composition | Concentration ranges of QAs (%)<sup>(d)</sup> | Analytical method LOD/LOQ if available | Comment e.g. other alkaloids present | Reference |
|------------------------|---------------------------------|----------------|---------------------------------------------|--------------------------------------|--------------------------------------|-----------|
| Lord                   | 1,020 mg/kg                     | Sparteine Lupinine 13α-OH-Lupanine Lupanine | 56 27 6.3 2.9                            | GC-NPD                               | From a Polish breeding station Gramine Ammodendrine 17-Oxosparteine | Chilomer et al. (2010) |
| Dukat, Mister, Taper   | 9–14 mg/kg<sup>(a)</sup>        | Sparteine 13α-OH-Lupanine Lupanine | 7.1-13.6 mg/kg < LOQ 1.1 mg/kg < LOQ 0.5 mg/kg | GC–MS                                | Grown in Italy                                    | Gresta et al. (2010) |
| Dukat, Taper, 2 x Mister, Nacional | 130–10,630 mg/kg | Sparteine Lupinine 13α-OH-Lupanine | 120-7,890 mg/kg 0-2,420 mg/kg Not-detected-10 mg/kg | GC-FID GC–IT-MS | Grown in Poland and Portugal 11,12-Dehydrolupanine Gramine | Magalhães et al. (2017) |
| Stated as sweet        | 500–895 mg/kg<sup>(b)</sup>    | Sparteine Lupinine | 2-50 mg/kg 3-10 mg/kg                        | GC-FID and GC–MS                     | Gramine (450-893 mg/kg)                                      | Reinhard et al. (2006) |
| Dukat, Mister, Taper (sweet) | 389–639 mg/kg<sup>(a)</sup> | Lupine<sup>(e)</sup> Sparteine Lupanine | 177-281 mg/kg 120-234 mg/kg 6.4-37 mg/kg | GC–MS                                | Grown in Italy Ammodendrine Feruloyllupinine l-Isosparteine | Romeo et al. (2018) |
| Not stated             | Not given                       | Lupine Sparteine Tetrahydrorhombifoline Lupanine | 60 30 < 1 < 1                           | GC–MS                                | Analysis of 56 lupin species for 100 QAs                      | Wink et al. (1995) |

FID: flame ionisation detector; GC–IT: gas chromatography–ion trap mass spectrometry; GC–MS: gas chromatography–mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; NPD: nitrogen-phosphorus detector; QA: quinolizidine alkaloids.

(a): Sum of individual QAs.
(b): Sum of alkaloids.
(c): Detector not reported.
(d): Given as percentage of total QA, unless another unit is given. In the latter case, the percentage of the individual QAs was not reported by the authors.
(e): Only results for QAs found in all varieties are shown in the paper.
| Variety/ cultivar/breed | Total QA concentration in seeds | QA composition | Concentration of QAs (%) | Analytical method | Comment e.g. other alkaloids present | Reference |
|------------------------|---------------------------------|----------------|--------------------------|-------------------|--------------------------------------|-----------|
| Not given              | 31,000 mg/kg DM                 | Lupanine, 13α-OH-Lupanine, Sparteine, Tetrahydrorhombifoline, 13α-Angeloyloxylupanine, 13α-Tigloyloxylupanine, α-Isolupanine, Multiflorine | 58, 15, 7.4, 3.5, 1.6, 0.3, 0.3, 0.14 | GC-MS and GC-NPD | From Peru 3β-OH-Lupanine<sup>a</sup>, 3β,13α-diOH-Lupanine<sup>b</sup> 13α-Benzyloxyulpamine 13α-Cinnamoyloxylupanine 6 QAs tentatively identified 3 un-identified QAs | Hatzold et al. (1983) |
| Not given              | Not given                       | Lupanine, Sparteine, 13α-OH-Lupanine, Tetrahydrorhombifoline, 13α-Angeloyloxylupanine, Angustifoline, 13α-Tigloyloxylupanine, α-Isolupanine, Multiflorine | 47, 16, 7, 2, 2, 1, 1, < 1, < 1 | GC-MS | 3β-OH-Lupanine, 12% of total Analysis of 56 lupin species for 100 QAs | Wink et al. (1995) |

QA: quinolizidine alkaloids; DM: dry matter; NPD: nitrogen-phosphorus detector; GC-MS: gas chromatography-mass spectrometry.

<sup>a</sup>: Reported by the authors under its synonym 4-OH-lupanine.

<sup>b</sup>: Reported by the authors under its synonym 4,13-diOH-lupanine.
Table D.5: Occurrence of quinolizidine alkaloids reported in overview papers

| Concentration of total alkaloids | QA composition                | Ranges of QAs (%) | References                               |
|----------------------------------|--------------------------------|-------------------|------------------------------------------|
| **L. albus**                     |                               |                   |                                          |
| Bitter varieties: 320–29,600 mg/kg DM | Lupanine | 57–70              | Pilegaard and Gry (2008)                 |
| Sweet varieties: < 100–4,200 mg/kg DM | α-isolupanine | < 1–25             |                                          |
|                                  | 13α-OH-lupanine | 0–20              |                                          |
|                                  | Multiflorine | 0–18              |                                          |
|                                  | Albine | < 0.1–15            |                                          |
|                                  | 11,12-seco-12,13-Didehydromultiflorine | < 0.1–13          |                                          |
|                                  | Angustifoline | < 1–8.9           |                                          |
|                                  | 13α-OH-Multiflorine | 1–1.5            |                                          |
|                                  | 13α-Tigloyloxylupanine | 0.5–1.4          |                                          |
|                                  | Sparteine | 0–0.7              |                                          |
|                                  | 13α-Angeloxyloxylupanine | 0.2–0.6          |                                          |
|                                  | 17-Oxolupanine | 0.2–0.4           |                                          |
| Not known: 370–8,050 mg/kg DM | Lupanine | 50–3,670 mg/kg DM |                                          |
|                                  | Albine | 15                |                                          |
|                                  | 13α-OH-Lupanine | 8                |                                          |
|                                  | Multiflorine | 3                |                                          |
| 50–3,670 mg/kg DM | Lupanine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
| Sweet: 50–500 mg/kg DM | Albine | 50                |                                          |
| Bitter: Up to 80,000 mg/kg in wild types | Lupanine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | Multiflorine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | 13α-OH-Lupanine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | Multiflorine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | 13α-Angeloxyloxylupanine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | Minor: Angustifoline | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | α-Isolupanine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | Sparteine | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
|                                  | Tetrahydrorhombifoline | 50–3,670 mg/kg DM | Carvajal-Larena et al. (2016) (a)       |
| **L. angustifolius**             |                               |                   |                                          |
| Bitter: 14,400–25,511 mg/kg DM | Lupanine | 24–72              | Pilegaard and Gry (2008)                 |
| Sweet: 20–1,918 mg/kg DM | 13α-OH-Lupanine | 12–52              |                                          |
| Not stated: 250–6,670 mg/kg DM | Isoangustifoline | 0.5–31             |                                          |
|                                  | Angustifoline | 1.9–16             |                                          |
|                                  | α-Isolupanine | < 1–15             |                                          |
|                                  | Sparteine | 0.03–5             |                                          |
|                                  | Tetrahydrorhombifoline | 0.03–5            |                                          |
|                                  | Other alkaloids | < 1–2.9            |                                          |
|                                  | 950–14,000 mg/kg seed DM | 0.03–5            |                                          |
|                                  | Lupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | 13α-OH-Lupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | Angustifoline | 950–14,000 mg/kg DM | BfR (2017a)                             |
| Sweet: < 500 mg/kg | Lupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | Angustifoline | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | 13α-OH-Lupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | Minor: Isoangustifoline | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | α-Isolupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | 17-Oxolupanine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | Sparteine | 950–14,000 mg/kg DM | BfR (2017a)                             |
|                                  | Tetrahydrorhombifoline | 950–14,000 mg/kg DM | BfR (2017a)                             |
| **L. luteus**                    |                               |                   |                                          |
| 4,700–15,000 mg/kg seed DM | Lupanine | 4,700–15,000 mg/kg DM | Carvalja-Larena et al. (2016) (a)       |
|                                  | Sparteine | 60                 |                                          |

www.efsa.europa.eu/efsajournal 91 EFSA Journal 2019;17(11):5860
| Concentration of total alkaloids | QA composition | Ranges of QAs (%) | References |
|---------------------------------|----------------|------------------|------------|
| Sweet: < 1,000 mg/kg           | Lupanine       | 60               | BfR (2017a) |
| Bitter: 5,000–20,000 mg/kg     | Spaerteine     | 30               |            |
|                                 | Minor:         |                  |            |
|                                 | Feruloyllupanine |                |            |
|                                 | 3β-Isosparteine  |                |            |
|                                 | Lupanine       |                  |            |
|                                 | 17-Oxosparteine |                  |            |
|                                 | Tetrahydrorhombifoline |      |            |
| **L. mutabilis**                |                |                  |            |
| 70–45,000 mg/kg seed DM        | Lupanine       | 13–85            | Carvajal-Larenas et al. (2016) |
|                                 | 3β-OH-Lupanine  | 6.6–19           |            |
|                                 | 3β-OH-Lupanime(b) | 1.6–15        |            |
|                                 | Tetrahydrorhombifoline | 1.1–8.7       |            |
|                                 | Angustifoline   | 2.0–3.5          |            |
|                                 | 3β,13α-diOH-Lupanine(c) | 0.6–5.4   |            |
|                                 | 13α-Angeloxyloxylupanine | 2.1       |            |
|                                 | cis-13α-       | 1.6–2.0          |            |
|                                 | Cinnamoyloxylupanine | 1.2        |            |
|                                 | Multiflorine    | 0.2–2.0          |            |
|                                 |                  | 0.1–1.8          |            |
| Sweet: < 1,000 mg/kg           | Lupanine       | 37–75            | BfR (2017a) |
| Bitter: 10,000–40,000 mg/kg    | 3β-OH-Lupanine  | 4–22             |            |
|                                 | 13α-OH-Lupanine  | 4–20             |            |
|                                 | Spaerteine      | 3–20             |            |
|                                 | Tetrahydrorhombifoline | 0.1–4        |            |
|                                 | Minor:          |                  |            |
|                                 | 13α-Angeloxyloxylupanine |        |            |
|                                 | Angustifoline   |                  |            |
|                                 | 11,12-Dehydrosparteine |          |            |
|                                 | 3,13-diOH-Lupanine |             |            |
|                                 | α-Isolupanine   |                  |            |
|                                 | Multiflorine    |                  |            |
|                                 | 17-Oxosparteine |                  |            |
|                                 | 13-Tigloyloxylupanine |              |            |
|                                 | Various esters of 13α-OH-lupanine, 3β-OH-lupanine and 3β,13α-diOH-lupanine | | |

DM: dry matter; QA: quinolizidine alkaloids.
(a): Same data as in Wink et al. (1995).
(b): Reported by the authors as 4-OH-lupanine.
(c): Reported by the authors as 4,13-diOH-lupanine.
Table D.6: Occurrence of quinolizidine alkaloids in leaves of *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis*

| Species     | Total concentration of QAs (mg/kg) | Quinolizidine alkaloids identified                                      | Contribution of individual QAs | Reference       |
|-------------|------------------------------------|-------------------------------------------------------------------------|--------------------------------|-----------------|
| *L. albus*  | Not stated(b)                      | Lupanine                                                                | 50%                           | Wink et al. (1995) |
|             |                                    | 13α-OH-Lupanine                                                         | 12%                           |                 |
|             |                                    | Multiflorine                                                            | 10%                           |                 |
|             |                                    | 13α-tigloyloxylupanine                                                  | 10%                           |                 |
|             |                                    | Albine                                                                  | 6%                            |                 |
|             |                                    | Angustifoline                                                           | 3%                            |                 |
|             |                                    | Sparteine                                                               | < 1%                          |                 |
|             |                                    | Tetrahydrohombifoline                                                  | < 1%                          |                 |
|             |                                    | 13α-Isolupanine                                                         | < 1%                          |                 |
|             |                                    | 13α-Angeloyloxylupanine                                                | < 1%                          |                 |
| *L. angustifolius* | Not stated                       | Lupanine                                                                | 33%                           | Wink et al. (1995) |
|             |                                    | 13α-OH-Lupanine                                                         | 20%                           |                 |
|             |                                    | Angustifoline                                                           | 20%                           |                 |
|             |                                    | Tetrahydrohombifoline                                                  | < 1%                          |                 |
|             |                                    | 13α-Isolupanine                                                         | < 1%                          |                 |
| *L. angustifolius* | 64–20,205(a)                    | 13α-Isolupanine                                                         | 4.5–9,478 mg/kg               | Philippi et al. (2016)(c) |
|             |                                    | 13α-OH-Lupanine                                                         | 20–3,433 mg/kg                |                 |
|             |                                    | Lupanine                                                                | 10–2,623 mg/kg                |                 |
|             |                                    | Angustifoline                                                           | 6.3–2,504 mg/kg               |                 |
|             |                                    | Tetrahydrohombifoline                                                  | 3.0–2,348 mg/kg               |                 |
|             |                                    | 13α-Tigloyloxylupanine                                                 | 7.2–1,029 mg/kg               |                 |
|             |                                    | Multiflorine                                                           | 0–40 mg/kg                    |                 |
|             |                                    | Sparteine                                                               | 0–26 mg/kg                    |                 |
| *L. luteus* | Not stated                        | Sparteine                                                               | 55%                           | Wink et al. (1995) |
|             |                                    | Lupinine                                                                | 20%                           |                 |
|             |                                    | Tetrahydrohombifoline                                                  | < 1%                          |                 |
|             |                                    | Lupanine                                                                | < 1%                          |                 |
| *L. mutabilis* | Not stated                   | Lupanine                                                                | 35%                           | Wink et al. (1995) |
|             |                                    | 13α-Tigloyloxylupanine                                                 | 25%                           |                 |
|             |                                    | 13α-OH-Lupanine                                                         | 9%                            |                 |
|             |                                    | Tetrahydrohombifoline                                                  | 7%                            |                 |
|             |                                    | 13α-Isolupanine                                                         | 7%                            |                 |
|             |                                    | 13α-Angeloyloxylupanine                                                | 3%                            |                 |
|             |                                    | Sparteine                                                               | 1%                            |                 |
|             |                                    | Angustifoline                                                           | < 1%                          |                 |
|             |                                    | 13α-Isolupanine                                                         | < 1%                          |                 |

(a): Concentration of total alkaloids.
(b): In Wink et al. (1995) the analysed cultivars are not stated and the total amount of QAs are also not given.
(c): The paper of Philippi et al. (2016) describes results for the analysis by GC-FID of leaves from 46 different varieties of *L. angustifolius*. 
### Appendix E – Identification and selection of relevant scientific literature and reports

#### Formation, Occurrence, Exposure

**Search terms**

```
TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermop sine OR multiflorine OR tetrahydrohombifoline OR OH-lupanine OR an gelyoxyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: (occurrence OR exposure OR levels OR concentration OR formation OR content OR food OR feed OR livestock)
```

**Numbers of papers** 5,741

#### ADME and Mode of action

**Search terms**

```
TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermop sine OR multiflorine OR tetrahydrohombifoline OR hydroxylupanine OR angeloyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: (toxicokinetic* OR metabolism OR distribution OR excretion OR absorption OR mode of action OR mechanism of action OR biotransformation OR elimination OR reduction OR detoxification OR activity OR genetic polymorphism OR poor metaboliser OR extensive metaboliser OR acetylcholine receptors OR sodium channel blocker OR potassium channel blocker OR antiarrhythmic OR uterus contract* OR oxytotic
```

**Numbers of papers** 4,347

#### Processing

**Search terms**

```
TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermop sine OR multiflorine OR tetrahydrohombifoline OR hydroxylupanine OR angeloyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: detoxification OR debittering OR processing OR soaking OR stability OR reduction
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**Numbers of papers** 1,948

#### Animal toxicity

**Search terms**

```
TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermop sine OR multiflorine OR tetrahydrohombifoline OR hydroxylupanine OR angeloyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: (toxicity OR toxic* OR acute OR subacute OR subchronic OR chronic OR mutagen* OR cancer* OR genotox* OR reprotox* OR nephrotox* OR neurotox* OR hepatotox* OR immunotox* OR haemotox* OR hematotox* OR cytotox* OR develop* toxicity OR teratogen* OR rat OR mouse OR animal* OR dog OR cow OR heifer OR calves OR sheep OR goats OR horse OR fish OR ruminant OR pig OR piglet OR swine OR sow OR livestock
```

**Numbers of papers** 3,050

#### Human observations

**Search terms**

```
TOPIC: (lupanine OR lupinine OR isolupanine OR isoangustifoline OR sparteine OR cytisine OR methylcytisine OR albine OR angustifoline OR isosparteine OR anagyrine OR thermop sine OR multiflorine OR tetrahydrohombifoline OR hydroxylupanine OR angeloyloxylupanine OR tigloyloxylupanine OR cinnamoyloxylupanine OR quinolizidine OR lupin*) AND TOPIC: (biomarker OR biological marker OR case stud* OR incidental poisoning OR poisoning OR human poisoning OR epidemic*)
```

**Number of papers** 195

**Data base used** Web of Science

**Total number** 8,545

**Considered relevant(a)** 37

(a): This is not the total number of relevant publications, but that of those considered as relevant in addition to those in the previous search (N = 175).
Appendix F – EFSA guidance documents on risk assessment

EFSA (European Food Safety Authority), 2007. Guidance of the Scientific Committee on a request from EFSA related to uncertainties in Dietary Exposure Assessment. EFSA Journal 2007;4(12):438, 54 pp. https://doi.org/10.2903/j.efsa.2007.438

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. EFSA Journal 2009;6(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557

EFSA (European Food Safety Authority), 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA (European Food Safety Authority), 2011. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal 2011;9(12):2490, 33 pp. https://doi.org/10.2903/j.efsa.2011.2490

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012. Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2012;10(1):2534, 26 pp. https://doi.org/10.2903/j.efsa.2012.2534

EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2012. Scientific Opinion on Risk Assessment Terminology. EFSA Journal 2012;10(5):2664, 43 pp. https://doi.org/10.2903/j.efsa.2012.2664

EFSA Scientific Committee, 2017. Update: use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 48 pp. https://doi.org/10.2903/j.efsa.2017.4658
Appendix G – Feed intakes and diet composition (livestock)

Information on the amount of total diet consumed per day, the inclusion rate of lupins in the diet and the concentration of QAs in the lupin seeds is needed in order to estimate daily exposure to quinolizidine alkaloids (QAs) by farm animals, horses and companion animals. Details of the feed intakes and live weights for different farm animals, horses and companion animals used to estimate exposures in this Opinion have been obtained from a number of different sources, details of which are given in the tables below. Inclusion rates for lupin seeds in diets of different livestock species have been obtained from publications in which maximum recommended levels have been published, in order to estimate total daily intake of lupins. These data have been combined with levels of QAs in lupin seeds (see Section 3.3.2 for details) to estimate daily exposure to QAs.

G.1. Feed intakes

G.1.1. Ruminants and horses

Dairy cows

The amounts of feed given to lactating dairy cows varies according to the amount and quality of forages and other feeds available, the weight of the cow and its milk yield. In this Opinion, it is assumed that non-forage (i.e. complementary) feeds are fed at the rate of 0.3 kg/kg of milk produced (Nix, 2010). Exposures to QAs have been estimated for a 650-kg dairy cow. Assumptions on the amounts of forages and non-forage feed are given in Table G.1.

Beef cattle

There are a wide variety of beef production and husbandry systems in Europe. They may be categorised broadly as forage-based or cereal-based systems, although combinations of these systems are commonly found. In this opinion, an exposure estimate has been made for a 500 kg animal with a daily dry matter intake of 12 kg (Table G.1).

Sheep and goats

Many breeds and systems of management have been developed for sheep and goats to suit the land, climate and husbandry conditions in the EU. As for other ruminants, forages may be the only feeds used after weaning (NRC, 2007a). Common exceptions to this are pregnant and lactating animals, whose feed is usually supplemented with non-forage feeds or manufactured compound (complementary) feeds (AFRC, 1993; NRC, 2007a). In this Opinion, exposure estimates have been made for lactating sheep and goats. The CONTAM Panel has used a daily dry matter intake of 2.8 kg for an 80-kg lactating sheep feeding twin lambs to estimate the exposures. For lactating goats, the CONTAM Panel has used a daily dry matter intake of 3.4 kg for a 60-kg goat for milking (4 kg milk/day). For fattening goats, a body weight of 40 kg and feed intake of 1.4 kg DM/day has been assumed (Table G.1).

Horses

Horses are non-ruminant herbivores. They typically consume 2–3.5% of their body weight in feed (dry matter) each day, of which a minimum of 50% should be as forage (pasture grass or hay) (NRC, 2007b). Assumed intakes are given in Table G.1.
**G.1.2. Non-ruminant animals**

**Pigs**

Although there is a considerable range of pig production systems in Europe, exposure estimates have been made for piglets (pig starter), finishing pigs and lactating sows (using feed intakes proposed by EFSA FEEDAP Panel, 2012). Details are given in Table G.2.

**Poultry**

The CONTAM Panel applied the live weights and feed intakes for fattening chickens (broilers), laying hens and turkeys proposed by EFSA FEEDAP Panel (2012) and for ducks by Leeson and Summers (2008) (Table G.2).

**Farmed fish (salmonids)**

Commercially reared species include Atlantic salmon, rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. In this Scientific Opinion exposures to QAs been made for farmed salmon. Details of the body weights and feed intakes used are given in Table G.2.

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**Table G.1:** Live weights, growth rate/productivity, dry matter intake for cattle, sheep, goats and horses, and the proportions of the diet as non-forage

| Animal species                  | Live weight (kg) | Growth rate or productivity | Dry matter intake (kg/day) | % of diet as non-forage feed | Reference               |
|---------------------------------|------------------|------------------------------|----------------------------|-----------------------------|-------------------------|
| Dairy cows, lactating<sup>(a)</sup> | 650              | 40 kg milk/day               | 25                         | 40                          | OECD (2013)             |
| Fattening cattle: beef<sup>(b)</sup> | 500              | 1 kg LWG/day                 | 12                         | 15                          | OECD (2013)             |
| Sheep: lactating                | 80               | Feeding twin lambs           | 2.8                        | 50                          | OECD (2013)             |
| Goats: milking                   | 60               | 6 kg milk/day                | 3.4                        | 65                          | NRC (2007a)             |
| Goats: fattening                 | 40               | 0.2 kg LWG/day               | 1.4                        | 65                          | NRC (2007a)             |
| Horses                           | 450              | Moderate activity            | 9.0                        | 50                          | NRC (2007b)             |

LWG: live weight.

<sup>(a):</sup> Months 2-3 of lactation.

<sup>(b):</sup> Housed castrated cattle, medium maturing breed.

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**Table G.2:** Live weights and feed intake for pigs, poultry (EFSA FEEDAP Panel, 2012), ducks (Leeson and Summers, 2008) and fish

| Species                         | Live weight (kg) | Feed intake (kg dry matter/day) | Reference                   |
|---------------------------------|------------------|---------------------------------|-----------------------------|
| Pigs: starter                   | 20               | 1.0                             | EFSA FEEDAP Panel (2012)    |
| Pigs: finishing                 | 100              | 3.0                             | EFSA FEEDAP Panel (2012)    |
| Pigs: lactating sows            | 200              | 6.0                             | EFSA FEEDAP Panel (2012)    |
| Poultry: broiler starters       | 0.7              | 0.075                           | Leeson and Summers (2008)   |
| Poultry: broilers<sup>(a)</sup> | 2                | 0.12                            | EFSA FEEDAP Panel (2012)    |
| Poultry: laying hens            | 2                | 0.12                            | EFSA FEEDAP Panel (2012)    |
| Turkeys: fattening turkeys      | 12               | 0.40                            | EFSA FEEDAP Panel (2012)    |
| Ducks: fattening ducks          | 3                | 0.14                            | Leeson and Summers (2008)   |
| Salmonids                       | 2                | 0.04                            | EFSA FEEDAP Panel (2012)    |

<sup>(a):</sup> Fattening chickens.

**Rabbits**

Feed intakes of 65–80 g/kg bw per day have been reported (Carabano and Piquer, 1998). For the exposure estimates, the CONTAM Panel have assumed a live weight of 2 kg, and a daily feed intake of 75 g/kg bw (derived from Carabano and Piquer, 1998).
Companion animals: dogs and cats

The amount of food consumed is largely a function of the mature weight of the animal, level of activity, physiological status (e.g. pregnancy or lactation) and the energy content of the diet. In this Scientific Opinion, the CONTAM Panel assumed body weights (kg) and feed intakes (g dry matter/day) for dogs and cats of 25/360 and 4/60, respectively (derived from NRC, 2006).

G.2. Inclusion rates of lupins in the diets of farm animals, horses and companion animals

It has not been possible to obtain, from feed manufacturers, typical inclusion rates of lupins in the diets of farm animals, horses or companion animals. Therefore, where possible published reported maximum levels of inclusion that resulted in no adverse effects on animal health or productivity have been used to establish possible ‘worst-case’ scenarios. It should be noted however that recommended inclusion levels vary according to authors, and where recommended levels vary widely a conservative approach has been adopted. For some livestock, the data have been reviewed and reported in Feedipedia, an EU/FAO online feed database, unless otherwise stated.

G.2.1. Cattle, sheep, goats and horses

While forages are essential feeds to ruminants and horses, they are normally supplemented with non-forage feeds such as cereals, cereal by-products, oilseed meals and by-products of human food production. Whole-crop lupins may be fed to livestock, as the sole crop or grown in combination with other forages and may be fed fresh or ensiled. However, no data are available on levels of QAs in the whole plant and, therefore, it is assumed that it makes no contribution to exposure.

Table G.3: Assumed inclusion rates (%) of lupin meal in the complementary (i.e. non-forage) feeds of ruminants and horses (El Otmani et al., 2013; OECD, 2013)

| Non-forage feed materials | Dairy cows | Beef cattle | Lactating sheep | Lactating goats | Fattening goats | Horses |
|---------------------------|------------|-------------|-----------------|----------------|----------------|--------|
| Lupin meal (%)            | 20(a)      | 25(a)       | 15(a)           | 20(a)          | 20(b)          | 10(a)  |
| % of non-forage feeds in the diet | 40 | 15 | 50 | 75 | 40 | 50 |

(a): OECD (2013).
(b): El Otmani et al. (2013).

G.2.2. Pigs and poultry

High levels of non-starch polysaccharides in lupin seeds restrict their use in pig and poultry diets. For pigs, OECD (2013) recommends maximum inclusion rates of 15% in diets for young and breeding pigs, and 20% for finishing (fattening) pigs. However, other authors have suggested that higher levels may be tolerated, and (Kim et al., 2011) have recommend maximum inclusion rates in grower diets of 20% and in fattener diets up to 35%.

Based on a review of published studies in which lupin seeds or meal were fed to poultry, Jeroch et al. (2016) recommended a maximum inclusion rate of 15% in diets of laying hens, broiler chickens, growing turkeys and fattening ducks and geese. Similar maximum inclusion rates have been proposed by Smulikowska et al. (2014), although Yule and McBride (1975) reported reductions in feed utilisation when broilers were fed diets containing 16% lupin meal (compared to 8%). Differences may have been due to the use of different lupin cultivars used.

G.2.3. Rabbits

Rabbits are usually fed a pelleted diet (in the form of complete feedingstuffs) consisting of dried forages, cereals and vegetable proteins supplemented with minerals, vitamins and trace elements.

The usual recommended inclusion rate in rabbit diets ranges from 10% to 20%, although higher levels have been used in experimental studies without adverse effects. No general guidelines on the maximum inclusion rates of lupins in rabbit diets have been identified. Volek and Marounek (2008)

40 www.feedipedia.org
reported a study in which fattening rabbits (from 37 to 79 days of age) were fed a diet containing 15% *L. albus* meal. No adverse effects on feed intake, growth rate or the health of the rabbits were noted. Therefore, in this Opinion, an inclusion rate of 15% lupin meal in the diet of fattening rabbits has been assumed.

**G.2.4. Farmed fish (salmonids)**

Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fishmeal and fish oils, although alternative sources of oil and protein, including lupin seeds, are increasingly replacing fish-derived feeds.

The digestibility of lupin meals is strongly influenced by the high levels of non-starch polysaccharides (NSP) within the grain, and since most aquaculture species lack the ability to deal with dietary NSP almost all of the NSP in lupins is defaecated. At high levels, it is also likely that NSP may act as an anti-nutrient, effectively acting like fibre and inhibiting the digestive process for other nutritionally important components of the feed. However, improvements in dry matter digestibility may be achieved with the removal of the lupin seed coat, which is concomitant with a reduction in the levels of NSP within the meals (Smith et al., 2000).

There is considerable variability in the maximum recommended inclusion level of lupin meals in diets for aquaculture species, with values ranging from 20% to 70% (De la Higuera et al., 1988; Robaina et al., 1995; Burel et al., 1998; Williams, 1998). Based on a review of the literature available at the time, Carter et al. (2002) reported that the addition of 20% dehulled lupin to a commercial extruded salmon feed produced excellent growth performance under commercial aquaculture conditions, and this value has been used to estimate the maximum exposure for salmon.

**G.2.5. Companion animals (dogs and cats)**

Limited information is available on recommended levels of lupin seed or meal in dog or cat feeds. Brown (2009) reported a study which concluded that inclusion of lupins in diets for dogs at levels greater than 15% ‘may be problematic’ due to the presence of non-starch polysaccharides in the seeds. The European Pet Food Industry Federation (FEDIAF41) has provided EFSA with information on typical inclusion levels of certain feed ingredients; although this did not include any reference to lupins, the formulations included up to 8% soybean meal in dry cat and dog food. Therefore, the CONTAM Panel has assumed an inclusion rate of 10% lupins in dog and cat feeds as an alternative to soybean meal, to account (in part) for the lower protein content of lupins while remaining within the limit proposed by Brown (2009).

**G.3. Calculated dietary exposure for farm animals, horses and companion animals**

Estimates of mean LB and UB dietary exposure to TotQAs are given below for farm animals, horses and companion animals based on occurrence data for lupin seeds from *L. angustifolius* and *L. albus* (Table G.4; *n* = 54), as well as from both plant species separately (Tables G.5 and G.6).

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41 The European Pet Food Industry Federation (FEDIAF), Personal communication by email, May 2016.
Table G.4: Estimates of mean LB and UB exposure to TotQAs\(^{a}\) (n = 54) are given below for farm animals, horses and companion animals

| Species          | Diet concentration (mg/kg) | Intake mg/day | Intake mg/kg bw |
|------------------|---------------------------|---------------|-----------------|
|                  | Mean | P75 | Mean | P75 | Mean | P75 |
| Dairy: high yielding |      |     |      |     |      |     |
| LB    | 31.5 | 28.2 | 652  | 583 | 1.00 | 0.90 |
| UB    | 31.5 | 28.2 | 652  | 583 | 1.00 | 0.90 |
| Beef: fattening  |      |     |      |     |      |     |
| LB    | 14.8 | 13.2 | 142  | 127 | 0.35 | 0.32 |
| UB    | 14.8 | 13.2 | 142  | 127 | 0.35 | 0.32 |
| Sheep: lactating |      |     |      |     |      |     |
| LB    | 29.5 | 26.4 | 82.6 | 74.0 | 1.03 | 1.23 |
| UB    | 29.5 | 26.4 | 82.7 | 74.0 | 1.03 | 1.23 |
| Goats: lactating |      |     |      |     |      |     |
| LB    | 59.0 | 52.8 | 201  | 180 | 3.34 | 2.99 |
| UB    | 59.1 | 52.8 | 201  | 180 | 3.35 | 2.99 |
| Goats: fattening |      |     |      |     |      |     |
| LB    | 31.5 | 28.2 | 47.2 | 42.3 | 1.18 | 1.06 |
| UB    | 31.5 | 28.2 | 47.3 | 42.3 | 1.18 | 1.06 |
| Horses |      |     |      |     |      |     |
| LB    | 19.7 | 17.6 | 177  | 158 | 0.39 | 0.35 |
| UB    | 19.7 | 17.6 | 177  | 158 | 0.39 | 0.35 |
| Pig starter    |      |     |      |     |      |     |
| LB    | 59.0 | 52.8 | 59.0 | 52.8 | 2.95 | 2.64 |
| UB    | 59.1 | 52.8 | 59.1 | 52.8 | 2.95 | 2.64 |
| Pig finisher   |      |     |      |     |      |     |
| LB    | 98.4 | 88.1 | 295  | 264 | 2.95 | 2.64 |
| UB    | 98.5 | 88.1 | 295  | 264 | 2.95 | 2.64 |
| Lactating sow  |      |     |      |     |      |     |
| LB    | 78.7 | 70.4 | 472  | 423 | 2.36 | 2.11 |
| UB    | 78.8 | 70.4 | 473  | 423 | 2.36 | 2.11 |
| Chickens for fattening | | | | | | |
| LB    | 59.0 | 52.8 | 7.09 | 6.34 | 3.54 | 3.17 |
| UB    | 59.1 | 52.8 | 7.09 | 6.34 | 3.54 | 3.17 |
| Laying hens    |      |     |      |     |      |     |
| LB    | 59.0 | 52.8 | 7.09 | 6.34 | 3.54 | 3.17 |
| UB    | 59.1 | 52.8 | 7.09 | 6.34 | 3.54 | 3.17 |
| Turkeys for fattening | | | | | | |
| LB    | 59.0 | 52.8 | 23.6 | 21.1 | 1.97 | 1.76 |
| UB    | 59.1 | 52.8 | 23.6 | 21.1 | 1.97 | 1.76 |
| Ducks for fattening | | | | | | |
| LB    | 59.0 | 52.8 | 8.26 | 7.40 | 2.75 | 2.47 |
| UB    | 59.1 | 52.8 | 8.27 | 7.40 | 2.76 | 2.47 |
| Salmonids     |      |     |      |     |      |     |
| LB    | 78.7 | 70.4 | 3.15 | 2.82 | 1.57 | 1.41 |
| UB    | 78.8 | 70.4 | 3.15 | 2.82 | 1.58 | 1.41 |
| Cats          |      |     |      |     |      |     |
| LB    | 39.4 | 35.2 | 2.36 | 2.11 | 0.59 | 0.53 |
| UB    | 39.4 | 35.2 | 2.36 | 2.11 | 0.59 | 0.53 |
| Dogs     |      |     |      |     |      |     |
| LB    | 39.4 | 35.2 | 14.2 | 12.7 | 0.57 | 0.51 |
| UB    | 39.4 | 35.2 | 14.2 | 12.7 | 0.57 | 0.51 |
| Rabbits  |      |     |      |     |      |     |
| LB    | 59.0 | 52.8 | 8.85 | 7.92 | 4.43 | 3.96 |
| UB    | 59.1 | 52.8 | 8.86 | 7.92 | 4.43 | 3.96 |

bw: body weight; LB: lower bound; P75: 75th percentile; TotQA: total quinolizidine alkaloids; UB: upper bound.

(a): Calculated as the sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine, α-isolupanine.
Table G.5: Estimates of mean LB and UB exposure to TotQAs(a) from *L. angustifolius* (n = 32) are given below for farm animals, horses and companion animals

| Species                  | Diet concentration (mg/kg) | Intake mg/day | Intake mg/kg bw |
|--------------------------|---------------------------|---------------|-----------------|
|                          | Mean | P75 | Mean | P75 | Mean | P75 |
| Dairy: high yielding     |      |     |      |     |      |     |
| LB                       | 38.0 | 43.1 | 787  | 892 | 1.21 | 1.37 |
| UB                       | 38.0 | 43.1 | 787  | 892 | 1.21 | 1.37 |
| Beef: fattening          |      |     |      |     |      |     |
| LB                       | 17.8 | 20.2 | 171  | 194 | 0.43 | 0.48 |
| UB                       | 17.8 | 20.2 | 171  | 194 | 0.43 | 0.48 |
| Sheep: lactating         |      |     |      |     |      |     |
| LB                       | 35.6 | 40.4 | 100  | 113 | 1.25 | 1.89 |
| UB                       | 35.7 | 40.4 | 100  | 113 | 1.25 | 1.89 |
| Goats: lactating         |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 242  | 275 | 4.04 | 4.58 |
| UB                       | 71.3 | 80.8 | 243  | 275 | 4.04 | 4.58 |
| Goats: fattening         |      |     |      |     |      |     |
| LB                       | 38.0 | 43.1 | 57.0 | 64.7| 1.43 | 1.62 |
| UB                       | 38.0 | 43.1 | 57.1 | 64.7| 1.43 | 1.62 |
| Horses                   |      |     |      |     |      |     |
| LB                       | 23.8 | 26.9 | 214  | 242 | 0.48 | 0.54 |
| UB                       | 23.8 | 26.9 | 214  | 242 | 0.48 | 0.54 |
| Pig starter              |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 71.3 | 80.8| 3.56 | 4.04 |
| UB                       | 71.3 | 80.8 | 71.3 | 80.8| 3.57 | 4.04 |
| Pig finisher             |      |     |      |     |      |     |
| LB                       | 119  | 135 | 356  | 404 | 3.56 | 4.04 |
| UB                       | 119  | 135 | 357  | 404 | 3.57 | 4.04 |
| Lactating sow            |      |     |      |     |      |     |
| LB                       | 95.0 | 108 | 570  | 647 | 2.85 | 3.23 |
| UB                       | 95.1 | 108 | 571  | 647 | 2.85 | 3.23 |
| Chickens for fattening   |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 8.55 | 9.70| 4.28 | 4.85 |
| UB                       | 71.3 | 80.8 | 8.56 | 9.70| 4.28 | 4.85 |
| Laying hens              |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 8.55 | 9.70| 4.28 | 4.85 |
| UB                       | 71.3 | 80.8 | 8.56 | 9.70| 4.28 | 4.85 |
| Turkeys for fattening    |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 28.5 | 32.3| 2.38 | 2.69 |
| UB                       | 71.3 | 80.8 | 28.5 | 32.3| 2.38 | 2.69 |
| Ducks for fattening      |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 10.0 | 11.3| 3.33 | 3.77 |
| UB                       | 71.3 | 80.8 | 10.0 | 11.3| 3.33 | 3.77 |
| Salmonids                |      |     |      |     |      |     |
| LB                       | 95.0 | 108 | 3.80 | 4.31| 1.90 | 2.16 |
| UB                       | 95.1 | 108 | 3.80 | 4.31| 1.90 | 2.16 |
| Cats                     |      |     |      |     |      |     |
| LB                       | 47.5 | 53.9 | 2.85 | 3.23| 0.71 | 0.81 |
| UB                       | 47.6 | 53.9 | 2.85 | 3.23| 0.71 | 0.81 |
| Dogs                     |      |     |      |     |      |     |
| LB                       | 47.5 | 53.9 | 17.1 | 19.4| 0.68 | 0.78 |
| UB                       | 47.6 | 53.9 | 17.1 | 19.4| 0.68 | 0.78 |
| Rabbits                  |      |     |      |     |      |     |
| LB                       | 71.3 | 80.8 | 10.7 | 12.1| 5.35 | 6.06 |
| UB                       | 71.3 | 80.8 | 10.7 | 12.1| 5.35 | 6.06 |

bw: body weight; LB: lower bound; P75: 75th percentile; TotQA: total quinolizidine alkaloids; UB: upper bound.

(a): Calculated as the sum of lupanine, 13α-OH-lupanine, angustifoline, multiflorine, 13α-tigloyloxylupanine, α-isolupanine.
| Species                  | Diet concentration (mg/kg) | Intake (mg/day) | Intake (mg/kg bw) |
|-------------------------|----------------------------|-----------------|-------------------|
|                         | Mean | P75  | Mean | P75  | Mean | P75  |
| Dairy: high yielding    | LB   | 27.5 | 28.7 | 570  | 595  | 0.88 | 0.92 |
|                         | UB   | 27.6 | 28.7 | 571  | 595  | 0.88 | 0.92 |
| Beef: fattening         | LB   | 12.90| 13.5 | 124  | 129  | 0.31 | 0.32 |
|                         | UB   | 12.93| 13.5 | 124  | 129  | 0.31 | 0.32 |
| Sheep: lactating        | LB   | 25.8 | 26.9 | 72.2 | 75.4 | 0.90 | 1.26 |
|                         | UB   | 25.9 | 26.9 | 72.4 | 75.4 | 0.90 | 1.26 |
| Goats: lactating        | LB   | 51.6 | 53.9 | 175  | 183  | 2.92 | 3.05 |
|                         | UB   | 51.7 | 53.9 | 176  | 183  | 2.93 | 3.05 |
| Goats: fattening        | LB   | 27.5 | 28.7 | 41.3 | 43.1 | 1.03 | 1.08 |
|                         | UB   | 27.6 | 28.7 | 41.4 | 43.1 | 1.03 | 1.08 |
| Horses                  | LB   | 17.2 | 18.0 | 155  | 162  | 0.34 | 0.36 |
|                         | UB   | 17.2 | 18.0 | 155  | 162  | 0.34 | 0.36 |
| Pig starter             | LB   | 51.6 | 53.9 | 51.6 | 53.9 | 2.58 | 2.69 |
|                         | UB   | 51.7 | 53.9 | 51.7 | 53.9 | 2.59 | 2.69 |
| Pig finisher            | LB   | 86.0 | 89.8 | 258  | 269  | 2.58 | 2.69 |
|                         | UB   | 86.2 | 89.8 | 259  | 269  | 2.59 | 2.69 |
| Lactating sow           | LB   | 68.8 | 71.8 | 413  | 431  | 2.06 | 2.16 |
|                         | UB   | 68.9 | 71.8 | 414  | 431  | 2.07 | 2.16 |
| Chickens for fattening  | LB   | 51.6 | 53.9 | 6.19 | 6.47 | 3.10 | 3.23 |
|                         | UB   | 51.7 | 53.9 | 6.20 | 6.47 | 3.10 | 3.23 |
| Laying hens             | LB   | 51.6 | 53.9 | 6.19 | 6.47 | 3.10 | 3.23 |
|                         | UB   | 51.7 | 53.9 | 6.20 | 6.47 | 3.10 | 3.23 |
| Turkeys for fattening   | LB   | 51.6 | 53.9 | 20.6 | 21.6 | 1.72 | 1.80 |
|                         | UB   | 51.7 | 53.9 | 20.7 | 21.6 | 1.72 | 1.80 |
| Ducks for fattening     | LB   | 51.6 | 53.9 | 7.22 | 7.54 | 2.41 | 2.51 |
|                         | UB   | 51.7 | 53.9 | 7.24 | 7.54 | 2.41 | 2.51 |
| Salmonids               | LB   | 68.8 | 71.8 | 2.75 | 2.87 | 1.38 | 1.44 |
|                         | UB   | 68.9 | 71.8 | 2.76 | 2.87 | 1.38 | 1.44 |
| Cats                    | LB   | 34.4 | 35.9 | 2.06 | 2.16 | 0.52 | 0.54 |
|                         | UB   | 34.5 | 35.9 | 2.07 | 2.16 | 0.52 | 0.54 |
| Dogs                    | LB   | 34.4 | 35.9 | 12.4 | 12.9 | 0.50 | 0.52 |
|                         | UB   | 34.5 | 35.9 | 12.4 | 12.9 | 0.50 | 0.52 |
| Rabbits                 | LB   | 51.6 | 53.9 | 7.74 | 8.08 | 3.87 | 4.04 |
|                         | UB   | 51.7 | 53.9 | 7.76 | 8.08 | 3.88 | 4.04 |

bw: body weight; LB: lower bound; P75: 75th percentile; TotQA: total quinolizidine alkaloids; UB: upper bound.
(a): Calculated as the sum of lupanine, 13α-OH-lupanine, angustifoline, multflorine, 13α-tigloyloxylupanine, α-isolupanine.
### Appendix H – Hazard identification and characterisation

#### H.1. Summary of acute studies with quinolizidine alkaloids

| Route of administration | Alkaloid/tested material | Species (N) | Dose (mg/kg bw) | Reference |
|--------------------------|--------------------------|-------------|-----------------|-----------|
| i.p.                     | Lupanine                 | Rats (12)   | LD₅₀ 180–192    | Gordon and Henderson (1951) |
|                          |                          | Wistar rats (5) | MLD(a) 177     | Petterson et al. (1987) |
|                          |                          | Guinea pigs (13) | LD₀ 210–225   | Gordon and Henderson (1951) |
|                          |                          | Mice (39)    | LD₁₀₀ 80       | Gordon and Henderson (1951) |
|                          |                          | Swiss mice (10) | ND 75–85      | Gordon and Henderson (1951) |
|                          |                          | Swiss mice (5) | 175           | Gordon and Henderson (1951) |
|                          | 13α-OH-Lupanine          | Wistar rats (5) (starved) | LD₀ 199      | Petterson et al. (1987) |
|                          |                          | Swiss mice (10) | 36            | Yovo et al. (1984) |
|                          |                          | Swiss mice (5) | 64            | Yovo et al. (1984) |
|                          |                          | 13α-OH-Lupanine | 250          | Pothier et al. (1998) |
|                          | 13α-OH-Lupanine          | 13α-OH-Lupanine | 100          | Pothier et al. (1998) |
|                          |                          | 13α-OH-Lupanine | 78           | Yovo et al. (1984) |
|                          |                          | 13α-OH-Lupanine | 27           | Yovo et al. (1984) |
|                          | Sparteine                | Wistar rats (5) (starved) | LD₀ 199      | Petterson et al. (1987) |
|                          |                          | Swiss mice (10) | 410          | Yovo et al. (1984) |
|                          |                          | Swiss mice (10) | 220          | Yovo et al. (1984) |
| PO                      | Lupanine                 | Wistar rats (5) | LD₀ 2,279     | Petterson et al. (1987) |
|                          |                          | Swiss mice (10) | 410          | Yovo et al. (1984) |
|                          |                          | Swiss mice (10) | 220          | Yovo et al. (1984) |
|                          | Sparteine                | Hartley Guinea pigs (5) | LD₀ 78      | Yovo et al. (1984) |
|                          |                          | Hartley Guinea pigs (5) | 27           | Yovo et al. (1984) |
|                          |                          | 13α-OH-Lupanine | 64           | Pothier et al. (1998) |
|                          | L. mutabilis seed extracts | Swiss mice (5) | 64           | Pothier et al. (1998) |
| PO                      | L. angustifolius seed extracts | Wistar rats (5) (fed) | LD₀ 2,279     | Petterson et al. (1987) |
|                          |                          | Wistar rats (5) (starved) | LD₀ 2,401    | Pothier et al. (1998) |

bw: body weight; i.p.: intraperitoneal; PO: per os; i.v.: intravenous; MLD: minimal lethal dose; LD: lethal dose; N: number of animals per group.

(a): Minimal lethal dose: Lowest amount of a substance that, when introduced into the body, may cause death to individual species of test animals under a defined set of conditions.

(b): LD₀: the maximal non-lethal dose represents the dose at which no individuals are expected to die. This is just below the threshold for lethality.

(c): LD₁₀₀: dose killing all the animals.

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42 [https://goldbook.iupac.org/html/M/M03933.html](https://goldbook.iupac.org/html/M/M03933.html)

43 [https://toxtutor.nlm.nih.gov/02-004.html](https://toxtutor.nlm.nih.gov/02-004.html)
### H.2. Effects of orally administered quinolizidine alkaloids in repeated dose studies in experimental animals

| Species (number animals/group) | Source of QA Dosage (mg/kg bw per day) | Duration | Outcome | Doses not associated with effects | Reference |
|-------------------------------|----------------------------------------|----------|---------|-----------------------------------|-----------|
| Charles River rats (12/group); sex unspecified | 20% lupin protein diet *L. albus* (510 mg alkaloids/kg seeds; 45 mg/kg bw per day) 20% lupin protein diet *L. luteus* (910 mg alkaloids/kg seeds; 81 mg/kg bw per day) Casein diet as control group | 112 days | Normal weight gain Normal weights for several organs | 45 mg/kg bw per day (only dose tested) 81 mg/kg bw per day (only dose tested) | Ballester et al. (1980) |
| Sprague-Dawley male and female rats (40/group) | 15% lupin protein diet *L. mutabilis* (water debittered seeds, 160 mg alkaloids/kg; equivalent to 14.4 mg/kg bw per day). Casein diet as control group | 84 days | Normal weight gain Normal weights for several organs Normal haematological parameters and clinical chemistry | 14.4 mg/kg bw per day (only dose tested) | Schoeneberger et al. (1987) |
| Sprague-Dawley males and female rats (20/group) | Diet (20% lupin protein) based on *L. angustifolius* flour spiked to provide 250, 1,050 and 5,050 mg alkaloids/kg diet (equivalent to 25, 105, 505 mg/kg bw/day). The reference diet contained a background level of 50 mg alkaloids/kg (equivalent to 4.5 mg/kg bw per day) | 90-98 days | Normal weight gain Normal weights for several organs Increase in relative liver weights in females (all doses) Some altered liver foci (both sexes) | ND | Butler et al. (1996) |
| Sprague-Dawley males and female rats (20/group) | Diet based on *L. angustifolius* flour containing 0, 100, 330, 1,000 and 5,000 mg alkaloids/kg diet (equivalent to 10, 33, 100 and 500 mg/kg bw per day). The control basal diets were commercial preparations +/- 4.5% maltodextrin | 90 days | Lower weight gains (both sexes, two highest doses) Increases in relative liver weights (both sexes, highest dose) No liver lesions | 100 mg/kg bw per day based on increased relative liver weight | Robbins et al. (1996) |
| Male Wistar rats (8/group) | Diet (10% lupin protein) based on *L. angustifolius* seeds (90, 110, 150 mg alkaloids/kg feed in Baron, Zeus and Wersal cultivars, respectively. These values are equivalent to 8.1, 9.9 and 13.5 mg alkaloids/kg bw per day | 28 days | Lower weight gain and food intake*(a)* Decreased liver weights Increased TG (all cultivars) Decreased ALT (Zeus and Wersal). Liver lesions | ND | Stanek et al. (2015) |

ALT: alanine transaminase; bw: body weight; LOAEL: lowest-observed-adverse-effect level; ND: not determined; NOAEL: no-observed-adverse-effect level; QA: quinolizidine alkaloids; TG: triacylglycerols.

*(a)*: The CONTAM Panel noted that previous experiments with 10% protein without DL-methionine supplementation showed that animals performed poorly.
H.3. Repeated dose toxicity studies of limited relevance for the risk assessment

Ballester et al. (1982) studied the toxic effects of a diet containing 51.8 g flour from *L. albus* (var. Multolopa) per 100 g diet (supplemented with 0.2% DL-methionine) in comparison to a control diet fed to groups of 20 male and 20 female Wistar rats for up to 9 months. The control diet contained the same protein levels (20%) obtained by defatted soybean flour, fishmeal and dried skimmed milk. The alkaloid concentration of the test diet was 0.025% (corresponding to 12.5 mg/kg bw per day). At 24 and 36 weeks the weight of several organs and their histology were examined. Haematological parameters (haemoglobin (Hb), haematocrit (HCT), leukocytes) and tests of liver functions (serum glutamic-pyruvate transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) were analysed. The test diet was well tolerated throughout the experiment, with food consumption and food conversion efficiency being similar in all dietary groups. The lupin feed had no adverse effects on growth, mortality, haematology or blood chemistry. In comparison to the control group, the treatment group showed a reduction in the relative liver weights. The histology of the liver, kidneys, brain, gonads and small intestine did not differ from the controls. The CONTAM Panel noted inconsistencies in the reporting of this study (discrepancies between the text/Table and the Abstract/Conclusions on the reduction of relative liver weights).

Long-term consumption (700 days) of diets based on *L. angustifolius* seeds (320 g/kg) was compared to a control diet (lactalbumin, maize and potato starch, glucose and maize oil) in male Hooded-Lister rats (Rowett strain) (27 and 39 animals, respectively) (Grant et al., 1995). The alkaloid concentration in the seeds was not reported. After 30, 250 and 700 days, animals were sacrificed and several organs were analysed (stomach, small intestine, caecum and colon, liver, kidney, adrenals, pancreas, spleen, thymus, lungs, heart, thyroid and muscles). Feed conversion efficiency was reduced in lupin-fed rats and body weights were significantly decreased at all time points when compared to controls. Growth was retarded over the initial 250 days and later on body weights gains were similar to controls. Consumption of the lupin-diet did not reduce net protein or lipid deposition in tissues. No significant difference in body composition was identified as a result of lupin seed consumption. Caecum and colon weights were significantly increased in lupin-fed rats (less prominent increases in stomach and small intestine). The CONTAM Panel noted that the lack of information on the alkaloid concentration of the seeds does not allow a toxicological evaluation of these data.

With the aim of investigating whether a lupin seed diet resulted in pancreatic enlargement, male Hooded Lister rats (48 animals) were fed a diet based on *L. angustifolius* seeds (320 g/kg) (no information on QA levels) and followed for 800 days. In comparison to the control diet (lactalbumin, maize and potato starch, glucose and maize oil), no pancreatic enlargement was observed (Grant et al., 1993).

The influence of the alkaloids and oligosaccharide concentration on the utilisation of casein diets was studied in male and female Wistar rats (27–30 days old, 8 animals/group) (Zdunczyk et al., 1998). Animals were fed with a casein diet supplemented with extracts of QAs and oligosaccharides from high- and low-alkaloid *L. albus* seeds (0.15 and 0.32 g/kg bw, respectively). Feed consumption and body weights were recorded for 4 weeks. Absorption of nutrients (glucose, methionine and phosphorus) in the small intestine was also determined by a controlled flow of perfusion fluid through the small intestine of anaesthetized rats. Finally, the effect of the alkaloid and oligosaccharide concentration on the nutritional value of lupin seeds was determined in a diet (supplemented with DL-methionine and L-tryptophan) in which lupin seeds were the sole protein source. The alkaloid concentration of 0.32 g/kg did not reduce feed intake or weight gains of a casein-based dietary intake and only slightly lowered the coefficient of crude protein digestibility (TD) in comparison to the control group. Decreased glucose and methionine absorption in the small intestine did not correlate with the alkaloid but with the oligosaccharide concentration. Different levels of alkaloids and oligosaccharides in the diets containing lupin seeds had no significant effect on feed intake, weight gains of the animals or TD coefficients. The CONTAM Panel noted that the design of this study was not suitable for evaluating possible toxic effects of QAs.

In an experimental model of type 2 diabetes mellitus, sparteine, lupanine and 2-thionosparteine were administered in non-diabetic and streptozotocin-induced diabetic Wistar rats (8 weeks old, 280–300 g bw, 10 animal/group) (Bobkiewicz-Kozlowska et al., 2007). Administration of sparteine and 2-thionosparteine (8 mg/kg) and lupanine (22.1 mg/kg) were by single i.p. injections. Blood glucose levels were estimated 60, 90 and 120 min after injection and insulin serum concentration at 120 min.
Lupanine did not exert hypoglycaemic effects in diabetic and non-diabetic animals. In contrast, injection of sparteine and 2-thionosparteine lowered blood glucose levels in diabetic rats and showed hypoglycaemic effects similar to the glibenclamide positive control. However, no increase in plasma insulin concentration was observed.
### H.4. Summary of genotoxicity studies on quinolizidine alkaloids

| Compound            | Source                                      | Test system                                      | Experimental system                  | Concentration/treatment | Result | Comments                                      | Reference                  |
|---------------------|---------------------------------------------|--------------------------------------------------|--------------------------------------|--------------------------|--------|-----------------------------------------------|----------------------------|
| Lupanine            | Purified by the authors from *L. termis*    | Bacterial reverse mutation assay (Ames test)     | *Salmonella Typhimurium* TA97, TA98, TA100, TA102, TA1535, TA1538 | 5, 20, 100 µg/plate + S9 100 µg/plate - S9 | Negative | No toxicity                                   | Santiago Quiles et al. (2010) |
| Extracts *L. termis*| Ethanol extraction from *L. termis*         | Bacterial reverse mutation assay (Ames test)     | *S. Typhimurium* TA97, TA98, TA100, TA102, TA1535, TA1538 | 5, 20, 100 µg/plate + S9 100 µg/plate - S9 | Negative | No toxicity                                   |                            |
|                     |                                             | Mouse lymphoma assay                              | L5178Y TK<sup>−/−</sup> cells          | 984, 1,161, 1,370, 1,617, 1,908, 2,251 µg/mL -S9: 24 h and 4 h +S9: 4 h | Negative | No significant toxicity at maximal solubility level (2,251 µg/mL) |                            |
|                     |                                             | Mouse micronucleus assay                          | Balb C mice                          | single i.p. injection: 635 mg/kg bw sampling time: 24 h and 48 h | Negative | Dose corresponding to 50% of LD<sub>50</sub> |                            |
| Sparteine           | Commercial                                  | SSBs by Comet assay                               | Tradescantia (clone 4430)            | 0.01, 0.1, 0.5, 1 mM    | **Positive:** Only at 0.5 mM | Non-standard test No dose response | Silva et al. (2014)         |
| Extracts *L. mexicanus* and *L. montanus* | Ethanol extraction                          | SSBs by Comet assay                               | Tradescantia (clone 4430)            | 0.01, 0.1, 0.5, 1 mM    | **Positive:** All doses | No dose response |                            |

bw: body weight; i.p.: intraperitoneal; SSB: single-strand break; LD<sub>50</sub>: lethal dose killing half of the animals.
H.5. **Studies on adverse effects in farm animals, horses and companion animals that are of limited relevance for the risk assessment**

**Cattle**

In a kinetic study by Gardner and Panter (1993; mentioned also in Section 3.1.1.2 on Toxicokinetics in farm animals, horses and companion animals), two cows of unspecified breed and age received a single oral dose (3 g/kg bw) of ground dried *L. caudatus* (aerial plant material) containing 19,200 mg QAs/kg, with lupanine (8%), 5,6-dehydrolupanine (22%), and anagyrine (22%) as the major QAs identified. The dose corresponded to 57.6 mg QAs/kg bw, including 4.8 mg lupanine/kg bw and 12.7 mg 5,6-dehydrolupanine/kg bw. Within 30 min, dosed animals showed protrusion of the nictitating membrane, frothing at the mouth, depression and slight incoordination of the hindquarter.

Cows of unspecified age, breed and weight with (group A, N = 6) or without (group B, N = 6) a history of delivering calves affected by lupin-induced arthrogryposis (also referred to as ‘crooked calf disease’) were orally gavaged with a single dose (2 g/kg bw) of dried and ground *L. leucophyllus*. The administered plant material contained 1,000 ± 100 mg/kg lupanine (i.e. 2.0 mg/kg bw), 3,900 ± 400 mg/kg 5,6-dehydrolupanine (i.e. 7.8 mg/kg bw), and 3,300 ± 300 mg/kg anagyrine (the total QA concentration was not reported) (Gay et al., 2004, see Section 3.1.1 on Toxicokinetics). One to 4 h after dosing, 7 out of 12 cows exhibited mild signs of toxicosis including uncoordinated gait, muscle fasciculations or coarse muscle tremors, and exaggerated response to external stimuli.

In a trial aimed at investigating the main TK parameters of lupin alkaloids in cattle cited above (Green et al., 2015a, see Section 3.1.1 on Toxicokinetics), four Holstein steers (289 ± 13 kg bw) received a single oral dose of dried ground *L. leucophyllus* plant material with calculated dosages of lupanine, 5,6-dehydrolupanine, anagyrine and a further unidentified alkaloid amounting to 17.2, 9.9, 12.9 and 6.7 mg/kg bw, respectively. No clinical adverse effects were reported. Likewise, six of each Angus and Holstein pregnant heifers did not exhibit clinical signs of intoxication following the oral administration of a single dose of the same dried ground *L. leucophyllus* as described above (Green et al., 2015a) corresponding to an equivalent dose of 7.6 mg/kg bw lupanine, 4.0 mg/kg bw 5,6-dehydrolupanine, 3.0 mg/kg bw unidentified alkaloid, and 5.7 mg/kg bw anagyrine (Green et al., 2015b).

**Sheep**

Lopez-Ortiz et al. (2004) investigated the effects of body conditions on the disposition of *L. argenteus* alkaloids (see Section 3.1.1). Ten Columbia ewes (age not mentioned) were administered with a single oral dose of dried seedpods (8.5 g/kg bw) containing 16,400 mg/kg total alkaloids (DM basis), which corresponded to 139 mg total alkaloids/kg bw. Besides three unidentified alkaloids, lupanine (8.2%), 5,6-dehydrolupanine (11.5%) and anagyrine (28.8%) were the main components. No clinical signs were observed at any time after dosing. Accordingly, no signs of intoxication were noticed in sheep following the single oral administration of ground dried *L. caudatus* (7.8 g/kg bw) containing 19,200 mg/kg QAs (Gardner and Panter, 1993; see Section 3.1.1), which amounted to approximately 150 mg/kg bw. Based on the available literature, sheep appear more resistant than cattle to the acute effects of QAs. In consideration of the higher blood levels and the longer persistence observed in the ovine species compared to the bovine one after a single QA administration, the apparent lower sensitivity to QA of sheep is likely not attributable to differences in kinetics.

**Goats**

No clinical signs of poisoning occurred in two goats after the single oral exposure to ground dried *L. caudatus* (7.8 g/kg bw) containing 19,200 mg/kg of QAs (Gardner and Panter, 1993; see Section 3.1.1).

**Pigs**

In an already mentioned study (see Section 3.1.1.2; Wasilewko et al., 1997), the effects of graded levels of dietary lupanine in pigs were compared to those from total QAs present in *L. albus*. Five piglets (unspecified sex and breed, initial body weight of 15 kg) were fitted with a post valvular...
T-shape caecum cannula and subjected to one of the following consecutive experimental treatments (washout period – if any – not specified): (i) a control barley-casein QA free diet for 7 days (control 1), (ii) a 9-day period on control diet plus increasing lupanine concentrations (100, 150 and 200 mg/kg diet, each three consecutive days), (iii) a lupin diet containing 200 mg/kg total QAs and (iv) the same control diet described above for 10 days (referred to as control 2). Blood sampling (fasted animals) was performed at the end of each treatment. Increase in blood AST activity, in the ratio AST/ALT, and in the bilirubin level were consistently recorded in the lupanine-treated animals; such changes are suggestive of liver impairment and were less pronounced in the animals fed with lupin QAs. This was also the case for haematological parameters (haemoglobin, haematocrit, mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC)). The authors concluded that the apparent lower toxicity of an equivalent amount of total QAs with respect to pure lupanine may be related to the lower concentration of lupanine, which represents 30–90% of total QA present in L. albus (Table 1). Due to a poor experimental design (treatment protocol, washout period not specified), the CONTAM Panel concluded that this study is unfit for deriving safe levels in pigs.

A study was carried out to establish safe dietary inclusion levels for different sweet lupin species (L. albus, L. angustifolius, L. luteus) and cultivars grown in Poland (Buraczewska et al., 1993). The total alkaloid concentration (estimated by TLC) in L. albus ranged from 600 to 950 mg/kg and from 400 to 510 mg/kg in L. angustifolius; in both species lupanine predominates over 13α-OH-lupanine and multiflorine. L. luteus showed a different picture, total alkaloids (ranging from 230 to 1,300 mg/kg) being composed mainly of lupinine, gramine (an indole-derivative), and sparteine. Piglets (N = 6, unspecified breed, weight range 20–45 kg) were offered a diet containing 12–35% lupins of different species and cultivars for 10–14 days. The total alkaloid concentration in the diet ranged between 88 and 180 mg/kg for L. angustifolius cultivars and around 120 mg/kg for L. albus cultivars and up to 455 mg/kg for L. luteus cultivars. As detected by feed intake depression, the tolerated total alkaloid levels of dietary inclusion differed according to the Lupinus species and cultivar. However, due to the poor experimental design, also including a restriction in feeding level, the CONTAM Panel decided not to consider this study.
### H.6. No-observed-adverse-effect levels, tolerated concentrations and doses of quinolizidine alkaloids in repeated dose studies in livestock

| Species (number of animals/group) | Source of QA/duration | Measured substance tested concentration in feed (mg/kg diet) | Tested parameters | Tolerated concentration (mg/kg diet) and endpoint | Tolerated dose (mg/kg bw per day) | Reference |
|----------------------------------|-----------------------|-------------------------------------------------------------|------------------|-----------------------------------------------|---------------------------------|-----------|
| Cattle (Jersey heifers) (6/group) | Low alkaloid cultivar of *L. albus* for 70 days Control diet based on soybean | Total alkaloids (QA composition not reported) Concentration: 131 mg/kg DM | Feed intake, weight gain, feed efficiency, serum biochemistry | 131 mg/kg DM for the lack of effects on feed intake, weight gain, feed efficiency, serum biochemistry | 4.5 mg/kg bw per day | Johnson et al. (1986) |
| Cattle (Friesian dairy cows) (5/group) | Intact (I) or heat detoxified (HD) lupin seeds (*L. albus*) at 150 or 300 g/kg diet DM for 70 days Control diet included sunflower meal as a protein source | The sum of the lupanine and 13α-OH-lupanine Concentrations: 0, 2,100, 3,800, 5,200, 8,000 mg/kg DM | Feed intake and milk yield | 2,100 mg/kg DM for reduced feed intake | 52 mg/kg bw per day | Mukisira et al. (1995) |
| Pigs (Large White x Landrace) (8/group (Exp 1) or 15/group (Exp 2)) | *L. angustifolius* seeds (mixture of ‘sweet’ cultivars) for 63 days (Exp 1) *L. angustifolius* seeds (‘bitter’ Fest cultivar) for 49 days (Exp 2) No control group | Total alkaloids (QA composition not reported) Concentrations: Exp 1: 120, 200, 280, 360, 440, 520 mg/kg diet (WW) Exp 2: 50, 200, 350 mg/kg diet (WW) Exp 1: 136, 227, 318, 409, 500, 591 mg/kg diet (DM) Exp 2: 57, 227, 398 mg/kg diet (DM) | Growth rate, feed intake, feed conversion ratio Liver and kidney gross and microscopic pathology (Exp 2 only) | 227 mg/kg DM for reduced growth rate and feed intake | 9 mg/kg bw per day | Godfrey et al. (1985) |
| Pigs (Polish Large White x Pietrain Barrows) (6/group) | *L. angustifolius* seeds of different cultivars (Wersal, Zeus, Baron) for 62 days Control diet based on soybean | Total alkaloids (QA composition not reported) Concentrations: 0, 97, 119, 156 mg/kg diet (DM) | Final body weight, daily weight gain, clinical signs and liver, kidney, pancreas and gastrointestinal gross and microscopic pathology | 119 mg/kg diet DM for reduction in daily weight gain | Dose calculation not possible | Rotkiewicz et al. (2007) |
| Species (number of animals/group) | Source of QA/duration | Measured substance tested concentration in feed (mg/kg diet) | Tested parameters | Tolerated concentration (mg/kg diet) and endpoint Tolerated dose (mg/kg bw per day) | Reference |
|----------------------------------|-----------------------|-------------------------------------------------------------|-------------------|---------------------------------------------------------------------------------|-----------|
| Pigs (10/group)                  | Raw and germinated seeds of *L. luteus* and *L. angustifolius* for 33 days Control diet based on soybean | Total alkaloids (Main QAs reported) Concentrations in *L. luteus*-diets: 10, 15, 17, 24 mg/kg diet (DM) Concentrations in *L. angustifolius*-diets: 18, 27, 31, 47 mg/kg diet (DM) | Final body weight, feed intake, average daily gain and feed conversion ratio, clinical signs, blood enzymes (the latter tested for some concentrations) | *L. luteus*: 24 mg/kg diet DM; no effects for zootechnical performance 1.5 mg/kg bw per day *L. angustifolius*: 18 mg/kg diet DM for reduced feed intake 1 mg/kg bw per day | Kasprowicz-Potocka et al. (2013) |
| Laying hens (12 or 30/group)    | Debittered lupin grist (16% in the ration) from one of the following: *L. albus* P, *L. albus* G, *L. mutabilis* for 168 days Control: no lupin inclusion | Total alkaloids (QA composition not reported) Concentrations: 1.1, 1.2, 6.2, 9.6 mg/kg diet (WW) 1.2, 1.3, 6.9, 10.7 mg/kg diet DM | Feed intake, feed efficiency, laying rate, egg weight, and egg fertility/hatchability | 10.7 mg/kg diet DM; no effects for any of the tested parameters 0.77 mg/kg bw per day | Vogt et al. (1987) |
| Single comb white leghorn laying hens (75–300/group) | Raw and processed seeds from *L. albus* cv. Ultra Up to 32 weeks Control: basal diet | Lupanine Concentrations in diets containing raw lupin seeds: 0, 8, 12, 16, 20, 24 mg/kg feed (a) | Mortality, feed consumption, egg production and egg weight | 16 mg/kg feed for egg weight and production 0.9 mg/kg bw per day | Watkins and Mirosh (1987) |
| Japanese quails (90/group)      | Raw, processed and extruded seeds of *L. albus* for 6 weeks Control: no lupin inclusion | Total alkaloids (> 80% lupanine) Concentration in diet containing raw lupin seeds: 4,771 mg/kg feed DM | Zootechnical performances (feed consumption, live weight, and feed conversion efficiency) | 4,771 mg/kg feed DM; no effects for any of the tested parameters 826 mg/kg bw per day | Arslan and Seker (2002) |
| Species (number of animals/group) | Source of QA/duration | Measured substance tested concentration in feed (mg/kg diet) | Tested parameters | Tolerated concentration (mg/kg diet) and endpoint Tolerated dose (mg/kg bw per day) | Reference |
|----------------------------------|-----------------------|------------------------------------------------------------|------------------|---------------------------------------------------------------------------------|-----------|
| **New Zealand White rabbits (16/group)** | Seeds from 2 different *L. albus* cultivars (extruded or not) for 80 days  
Control: no lupin inclusion | Total alkaloid (QA composition not reported)  
Group I: 0, 460 and 930 mg/kg DM  
Group A: 0, 430 and 860 mg/kg DM  
Group AE: 0, 130 and 260 mg/kg DM | Zootechnical performances (growth rate, feed intake, feed efficiency) | Group I: 460 mg/kg feed DM for feed consumption, final weight and daily weight gain  
30 mg/kg bw per day  
Group A: 430 mg/kg feed DM for final weight and daily weight gain  
30 mg/kg bw per day  
Group AE: 260 mg/kg feed DM; no effects for any of the tested parameters  
19 mg/kg bw per day | Battaglini et al. (1991) |
| **Rainbow trout (10/group)** | Pure lupinine added to the diet for 60 days | Pure lupinine  
Concentrations: 0, 53, 79, 107, 264, 532 or 1,071 mg/kg DM | Weight gain, feed intake, feed efficiency organ somatic index and organ histology | 107 mg/kg feed DM for reduced feed intake, growth rate and hepatosomatic index  
1.1 mg/kg bw per day (NOAEL) | Serrano et al. (2011) |
| **Juvenile rainbow trout (16/group)** | Pure sparteine added to the diet for 62 days | Pure sparteine  
Concentrations: 0, 54, 108, 269, 538, 1,073, 2,662 or 5,482 mg/kg DM | Weight gain, feed intake, feed efficiency organ somatic index and organ histology | 108 mg/kg feed DM for reduced body weight, weight gain, feed intake and hepatosomatic index  
1.4 mg/kg bw per day (NOAEL) | Serrano et al. (2012) |

bw: body weight; DM: dry matter; Exp: experiment; NOAEL: no-observed-adverse-effect level; QA: quinolizidine alkaloids; WW: wet weight.  
(a): Unclear in the paper whether this is expressed on DM basis.
Annex A – Dietary surveys and occurrence data in food submitted to EFSA
    Annex A can be found as a separate document at: https://doi.org/10.5281/zenodo.3471613.

Annex B – Occurrence data in feed submitted to EFSA
    Annex B can be found as a separate document at: https://doi.org/10.5281/zenodo.3471613.

Annex C – Results of acute dietary exposure assessment to quinolizidine alkaloids
    Annex C can be found as a separate document at: https://doi.org/10.5281/zenodo.3471613.